ORIGINAL SUBMISSION

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GRN 000515

April 15, 2014

Dr Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Dr Gaynor:

Re: GRAS Exemption Claim for (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized as Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting one hard copy and one electronic copy (on CD), as the notifier [TdeltaS Limited, University of Oxford, Parks Road, Oxford, United Kingdom], a notice of the determination, on the basis of scientific procedures, that (R)-3-hydroxybutyl (R)-3-hydroxybutyrate (D-β-hydroxybutyrate ester), as defined in the enclosed documentation, is GRAS under specific conditions of use as a food ingredient for use in foods consumed exclusively by high-performance athletes and individuals undergoing high energy expenditure and, therefore, is exempt from the pre-market approval requirements of the *Federal, Food, Drug and Cosmetic Act*. Information setting forth the basis for the GRAS determination, which includes detailed information on the notified substance and a summary of the basis for the GRAS determination, as well as a consensus opinion of an independent panel of experts in support of the safety of D-β-hydroxybutyrate ester under the intended conditions of use, also are enclosed for review by the Agency.

The enclosed electronic file for the Notice entitled, "GRAS Exemption Claim for (R) 3 hydroxybutyl (R)-3-hydroxybutyrate" was scanned for viruses prior to submission and is thus certified as being virus-free using McAfee VirusScan 8.8.

Should you have any questions or concerns regarding this GRAS Notification, please do not hesitate to contact me at any point during the review process, so that we may provide a response in a timely manner.

Sincerely,

(b) (6)

Professor Kieran Clarke
TdeltaS Limited
University of Oxford, UK

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30 Upper High Street
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(b) (6)

Professor Kieran Clarke
TdeltaS Limited
University of Oxford, UK

GRAS Exemption Claim for (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate

GRN 000515

Submitted for:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835
USA

Submitted by:

TΔS Limited
University of Oxford
Parks Road, Oxford
United Kingdom
OX1 3PT

April 11, 2014

GRAS Exemption Claim for (*R*)-3-hydroxybutyl (*R)*-3-hydroxybutyrate

Submitted for:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835
USA

Submitted by:

TΔS Limited
University of Oxford
Parks Road, Oxford
United Kingdom
OX1 3PT

April 11, 2014

GRAS Exemption Claim for (*R*)-3-hydroxybutyl (*R)*-3-hydroxybutyrate

Table of Contents

List of Appendices

List of Figures and Tables

I GRAS EXEMPTION CLAIM

I.A Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997) (U.S. FDA, 1997)]

(*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate (D-β-hydroxybutyrate ester) has been determined to be Generally Recognized as Safe (GRAS) by TΔS Limited (TΔS) for use as a food ingredient in the United States (U.S.), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections. Therefore, the use of D-β-hydroxybutyrate ester in foods as described below is exempt from the requirement of premarket approval.

Signed,

(b) (6)

Friday 11 April 2014

Professor Kieran Clarke Date

I.B Name and Address of Notifier

Professor Kieran Clarke
TdeltaS Limited
DPAG, Sherrington Building
University of Oxford
Parks Road, Oxford
United Kingdom
OX1 3PT

I.C Common Name of the Notified Substance

The common name of the notified substance is D-β-hydroxybutyrate ester.

I.D Conditions of Intended Use in Food

I.D.1 Intended Uses of D-β-Hydroxybutyrate Ester and Levels of Use

TΔS proposes to market D-β-hydroxybutyrate ester in selected categories of products (beverages, bars, and gels) designed exclusively for high-performance athletes and individuals undergoing extreme energy expenditure. D-β-hydroxybutyrate ester will be added to these products to result in intakes that will not exceed 0.36 g/kg body weight/serving. It is envisaged that a maximum of 2 to 3 servings per day will be consumed, resulting in a maximum daily intake of 1.1 g/kg body weight. Based on an average 70 kg adult, this maximum will relate to 75 g D-β-hydroxybutyrate ester per day.

It is proposed that for sport bars and gels, maximum use levels will relate to a use-level of approximately 30% (*i.e.*, 30 g per 100 g). When this is related to typical serving sizes of these products, bars will contain 11 g D-β-hydroxybutyrate ester per 40 g bar and gels will contain 18 g D-β-hydroxybutyrate ester per 60 g gel. For beverages, based on a maximum potential intake of 75 g D-β-hydroxybutyrate ester per day and 3 servings per day, this will relate to a content of 25 g per beverage, irrespective of volume. If related to a 240 mL beverage serving, D-β-hydroxybutyrate ester will have a use-level of approximately 15% in beverages. However, it is anticipated that D-β-hydroxybutyrate ester-containing beverages will be available in small volumes of approximately 60 to 80 mL, resulting in approximate use-levels of 30 to 40%.

I.D.2 Estimated Consumption of D-β-Hydroxybutyrate Ester Based upon Intended Uses

As D-β-hydroxybutyrate ester-containing products will be used as specialized products for consumption by high-performance athletes during exercise rather than as conventional foods for use by the general population, intakes of D-β-hydroxybutyrate ester were calculated on the basis of the proposed use-levels of the ingredient in each product category and the directions for use. A detailed intake assessment using U.S. food consumption datasets was not conducted because the ingredient will not be consumed by the general population.

D-β-Hydroxybutyrate ester intakes were calculated based on the proposed use levels for bars, gels, powder (sachet), and liquids and possible combinations of these products following the directions for use of 2 to 3 servings per day. Estimated intakes on a per kilogram body weight basis were calculated using an average adult body weight of 70 kg.

With 2 servings per day, the highest estimated intake of D-β-hydroxybutyrate ester would be from 2 beverages (liquid or powder) per day (2 servings x 25 g/serving = 50 g/day or 0.8 g/kg body weight/day), and the lowest estimated intake would be from 2 bars per day (2 servings x 11 g/serving = 22 g/day or 0.31 g/kg body weight/day).

With 3 servings per day, the highest estimated intake of D-β-hydroxybutyrate ester would be from 3 beverages (liquid or powder) per day (3 servings x 25 g/serving = 75 g/day or 1.2 g/kg body weight/day), and the lowest estimated intake from 3 bars per day (3 servings x 11 g/serving = 33 g/day or 0.47 g/kg body weight/day).

Based on the specialized use of D-β-hydroxybutyrate ester as an energy source during exercise in high-performance athletes rather than use in conventional foods by the general population, these values are considered to be reasonable estimates of consumption. The high cost and sharp, bitter taste of products containing D-β-hydroxybutyrate ester will deter the general population from using these products for general energy purposes. D-β-Hydroxybutyrate ester does not have stimulant properties and, as the caloric value of D-β-hydroxybutyrate ester is 4.7 kcal/g, consumption of the ingredient by individuals will only result in an increased caloric intake if not exercising.

I.E Basis for the GRAS Determination

Pursuant to Title 21, Section 170.30 of the Code of Federal Regulations (CFR) (U.S. FDA, 2013), D-β-hydroxybutyrate ester has been determined to be GRAS on the basis of scientific procedures. This GRAS determination is based on data generally available in the public domain pertaining to the safety of D-β-hydroxybutyrate ester, as discussed herein, and on consensus among a panel of experts who are qualified by scientific training and experience to evaluate the safety of the D-β-hydroxybutyrate ester ingredient as a component of food [see Appendix A, entitled “Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of (*R*)-3-Hydroxybutyl (*R*)-3-Hydroxybutyrate for Use as a Food Ingredient”].

At the request of TΔS, an Expert Panel (“the Expert Panel”) of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and to determine whether the intended uses of D-β-hydroxybutyrate ester in foods are safe and suitable and would be GRAS based on scientific procedures.

The Panel consisted of the following qualified scientific experts: Professor Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Professor Robert J. Nicolosi (University of Massachusetts Lowell), and Professor John A. Thomas, Ph.D. (Indiana University School of Medicine).

The Expert Panel convened on behalf of TΔS independently and collectively, and critically evaluated the data and information summarized herein and concluded that the intended uses in traditional foods described herein for D-β-hydroxybutyrate ester, meeting appropriate food-grade specifications and manufactured according to current Good Manufacturing Practice (cGMP), are safe, suitable, and GRAS based on scientific procedures. It also is the Expert Panel’s opinion

that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion.

D-β-Hydroxybutyrate ester is GRAS based on scientific procedures for its intended use as a food ingredient; therefore, it is excluded from the definition of a food additive, and thus, may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

I.F Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

TΔS Limited
DPAG, Sherrington Building
University of Oxford
Parks Road, Oxford
United Kingdom
OX1 3PT

Should FDA have any questions or additional information requests regarding this notification, TΔS will supply these data and information.

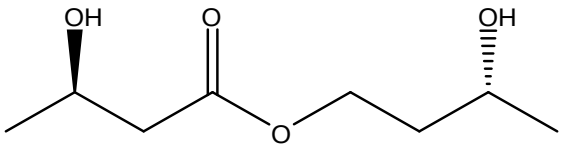
II. DETAILED INFORMATION ABOUT THE SOURCE AND IDENTITY OF THE SUBSTANCE

II.A Source and Identity

Molecular formula: $C_8H_{16}O_4$

Molecular weight: 176

Structure



Physical form: Colorless oil

Taste: Slight bitter, sharp taste; no odor

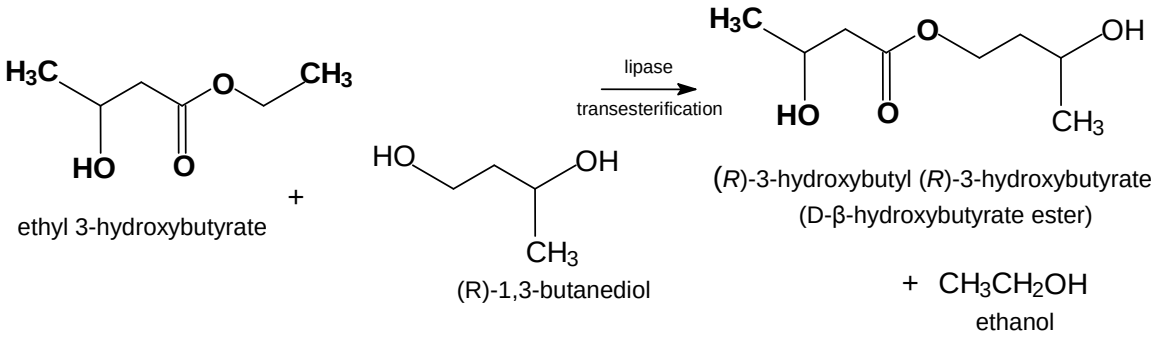
Enantiomeric Excess:	>99% (*R*)-1,3 butanediol (Diacel) >99% Ethyl (*R*) 3-hydroxybutyrate (Julich)	
Density:	1.0731 g/mL at 22°C	
Boiling point:	145°C at 1.8 Torr (Pope Scientific, July '07)	
Storage:	Room temperature	
Optical activity product:	[α]23.4/D	-46.2°, c = 1 in water
	[a]23.4/D	-38.7°, c = 1 in ethanol

II.B Method of Manufacture

The D-β-hydroxybutyrate ester is produced *via* an enzyme-catalyzed transesterification reaction of ethyl-D-hydroxybutyrate and (R)-1,3-butanediol (see Figure II.B-1). An ethyl ester of D-β-hydroxybutyrate (chemically synthesized) and (R)-1,3-butanediol are reacted together in the presence of immobilized lipase under mild vacuum to remove the resultant ethanol by-product. Wiped film distillation is employed as the last step, removing any remaining ethanol.

Both starting materials are food-grade (>99% purity). The lipase preparation used to catalyze the transesterification reaction is produced from *Aspergillus niger* genetically modified to express a lipase gene from *Candida antarctica*. This lipase preparation, produced by Novozyme, is commonly used in food production and complies with the recommended purity specifications for food-grade enzymes defined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemical Codex (FCC). In 2004, Novozyme filed a GRAS notice for a lipase preparation produced by *A. niger* expressing a gene encoding a lipase from *C. antarctica*, for use as an enzyme in the production of triglyceride products, with no questions from the FDA (GRN No. 158 – U.S. FDA, 2005).

Figure II.B-1 Schematic Overview of the Manufacturing Process for D-β-Hydroxybutyrate Ester



II.C Specifications and Analytical Data

D-β-Hydroxybutyrate ester is produced in accordance with cGMP, and in order to ensure a consistent and safe product, TΔS has established food-grade specification parameters for the final ingredient. The product specifications for D-β-hydroxybutyrate ester are presented in Tables II.C-1 and II.C-2.

Table II.C-1 Chemical Specifications for D-β-Hydroxybutyrate Ester

Specification Parameter	Specification	Method
D-β-hydroxybutyrate ester content	≥97.5%	GC/MS
Ethyl R 3-hydroxybutyrate content	<0.5%	GC/MS
R-1,3-butanediol content	<2.0%	GC/MS
Heavy Metals		
Arsenic (As)	<0.1 mg/kg	UD031
Lead (Pb)	<0.005 mg/kg	UD032:ICPMS/005
Cadmium (Cd)	<0.005 mg/kg	UD033:ICPMS/005

GC/MS, gas chromatography/mass spectrometry; ICPMS, inductively coupled plasma mass spectrometry

Table II.C-2 Microbiological Specifications for D-β-Hydroxybutyrate Ester

Specification Parameter	Specification (CFU/mg)	Method
Escherichia coli	< 5	HPA Standard Method F17, issue 2.4 May 2005
Moulds	< 10	BS 4285-3.6: 1986
Yeasts	< 10	BS 4285-3.6: 1986

BS = British Standards; CFU = colony forming unit; HPA = Health Protection Agency

Analyses of 3 lots of D-β-hydroxybutyrate ester confirm that the manufacturing process results in a product that is consistent and complies with product specifications (Table II.C-3). The

analytical data also demonstrate the absence of chemical impurities or microbiological contamination. Complete certificates of analysis for these lots are provided in Appendix B.

Table II.C-3 Summary of the Chemical Product Analysis for 3 Consecutive Lots of D-β-Hydroxybutyrate Ester

Specification Parameter	Specification	Manufacturing Lot		
		Batch 7	Batch 8	Batch 9
D-β-Hydroxybutyrate ester[1]	≥ 97.5%	98.8%	97.7%	98.2%
Ethyl R 3-hydroxybutyrate	< 0.5%	0.1%	0.1%	0.1%
R 1,3-Butanediol	< 2.0%	0.7%	1.4%	0.8%
Total	100.0%	99.6%	99.2%	99.1%
Heavy Metals				
Arsenic (As)	< 0.1 mg/kg	0.012 mg/kg	0.013 mg/kg	0.011 mg/kg
Lead (Pb)	< 0.005 mg/kg	< 0.005 mg/kg	< 0.005 mg/kg	< 0.005 mg/kg
Cadmium (Cd)	< 0.005 mg/kg	< 0.001 mg/kg	< 0.001 mg/kg	< 0.001 mg/kg

[1] "D-β-Hydroxybutyrate ester content" represents (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate, 3-betahydroxybutryl 1,3-butanediol monoester, and β-hydroxybutyrate 1,3-butanediol diester. These esters were grouped together in the product specifications and analyses because they are handled in the same manner in the body.

As shown in Table II.C-3, over 99% of the material is accounted for by D-β-hydroxybutyrate ester and its starting materials. The remaining percentage (less than 1%) not accounted for in the batch analyses is due to the derivatizing reagent used for analytical identification purposes. The derivatizing reagent is not present in the neat compound, as demonstrated by the presence of small peaks in the chromatographic analysis of the reagent alone. These very minor compounds can be subtracted from the chromatographic peak profiles, resulting in purity that approaches 100%. It also should be noted that there is variability in the gas chromatography/mass spectrometry measurements.

Microbiological analyses for D-β-hydroxybutyrate ester are presented in Table II.C-4.

Table II.C-4 Summary of the Microbiological Product Analysis for 5 Lots of D-β-Hydroxybutyrate Ester

Specification Parameter	Specification (CFU/mg)	Manufacturing Lot				
		Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Escherichia coli (CFU/mg)	<5	<5	<1	<1	<5	<1
Moulds (CFU/mg)	<10	<10	<1	<1	<10	<1
Yeasts (CFU/mg)	<10	<10	<1	<1	<10	<1

CFU = colony forming unit

During the synthesis of D-β-hydroxybutyrate ester, ethanol is produced as a by-product; however, ethanol is removed *via* vacuum. Three lots of D-β-hydroxybutyrate ester were analyzed using mass spectrometry for residual ethanol; the results of these analyses (Table II.C-5) demonstrate that the levels of residual ethanol are negligible and likely would not have

been detected using methods other than mass spectrometry. Moreover, according to the International Conference on Harmonisation (ICH) guidelines for residual solvents in pharmaceuticals for human use, ethanol is considered a solvent with low toxic potential and residual ethanol at levels of 0.5% are acceptable without justification (ICH, 2011).

Table II.C-5 Analysis of D-β-Hydroxybutyrate Ester for Residual Ethanol

Manufacturing Lot	Residual Ethanol (%)
Lot 7	0.03
Lot 8	0.06
Lot 9	0.08

II.D Stability

The stability of the D-β-hydroxybutyrate ester mixed with water (1:1) at different temperatures and different pH levels was assessed over a 330-day period. As shown in Figures II.D-1 to II.D-3, D-β-hydroxybutyrate ester generally remained stable when stored at temperatures ranging from 4 to 37°C and pH levels ranging from 3 to 10 throughout the storage period (shown in hours).

Figure II.D-1 Stability of D-β-Hydroxybutyrate at 4°C and pH Levels of 3, 7, and 10

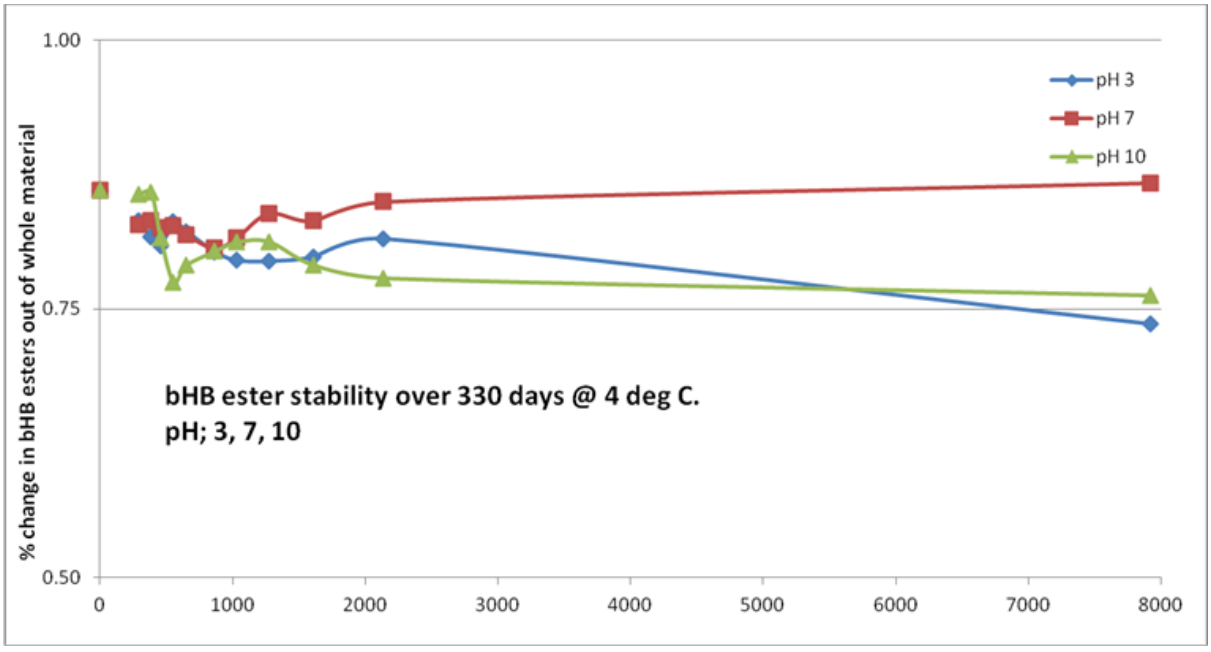


Figure II.D-2 **Stability of D-β-Hydroxybutyrate at Room Temperature and pH Levels of 3, 7, and 10**

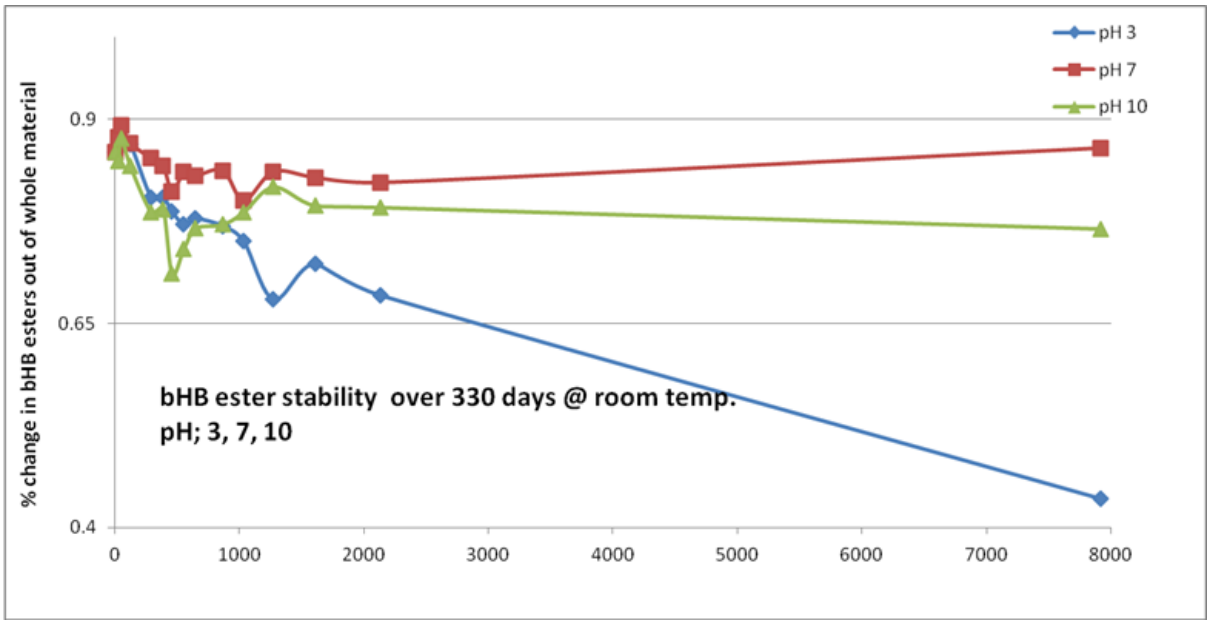
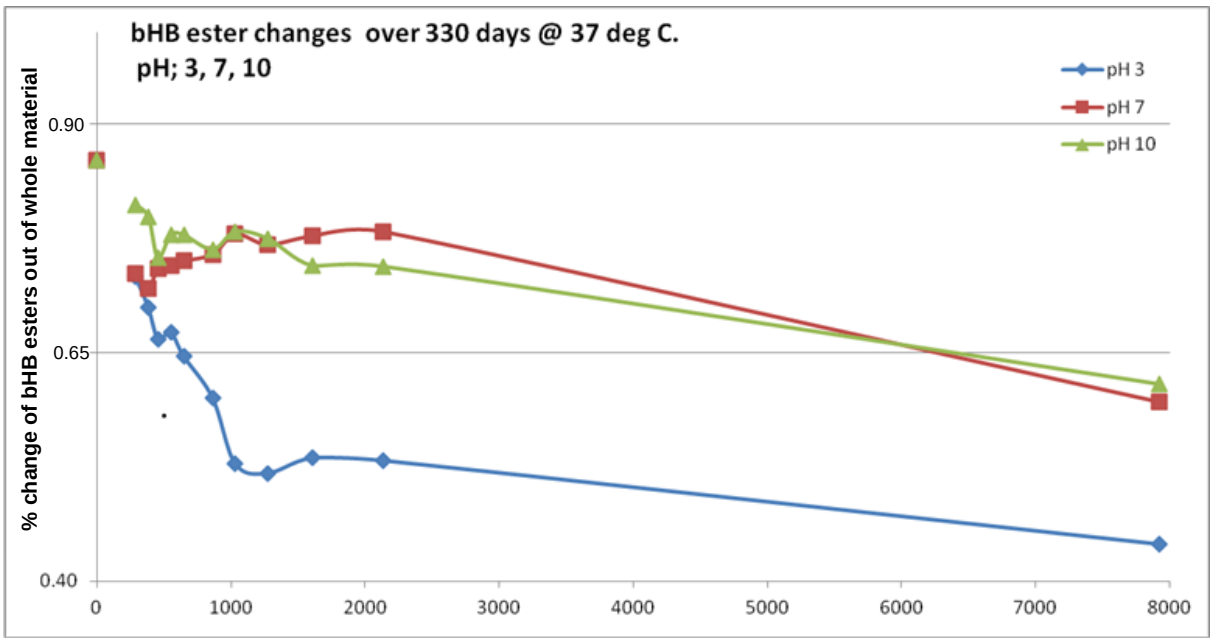


Figure II.D-3 **Stability of D-β-Hydroxybutyrate at Elevated Temperatures (37°C) and pH Levels of 3, 7, and 10**



III. SELF-LIMITING LEVELS OF USE

The use of D-β-hydroxybutyrate ester will be limited by its sharp, bitter taste.

IV. BASIS FOR GRAS DETERMINATION

IV.A Documentation to Support the Safety of D-β-hydroxybutyrate ester

The determination that D-β-hydroxybutyrate ester is GRAS is based on scientific procedures, and the information supporting the general recognition of the safety of the ingredient includes:

- Results of studies conducted on the ingredient, specifically, a 28-day toxicity study in rats, a 66-day rat study that included selected safety parameters, a developmental toxicity study in rats, and 2 human studies in which subjects consumed D-β-hydroxybutyrate ester for 1 or 5 days.
- The hydrolysis of D-β-hydroxybutyrate ester to D-β-hydroxybutyrate and (R)-1,3-butanediol in the gut, with the latter being further metabolized to D-β-hydroxybutyrate and acetoacetate in the liver, following ingestion. Thus, data on each of the metabolites also support of the safety of D-β-hydroxybutyrate ester.
- Corroborative evidence from animal feeding trials on the related compound 1,3-butanediol mono- and diacetoacetate.
- The endogenous production of the ketones D-β-hydroxybutyrate and acetoacetate, which is a normal homeostatic mechanism results in the increased production during times of fasting or limited glucose availability, demonstrating that the human body has the capacity to handle large amounts of ketones.
- The long history of using ketogenic diets to elevate blood ketone levels, similar to D-β-hydroxybutyrate ester.

Moreover, these data were reviewed by a panel of experts, qualified by scientific training and experience to evaluate the safety of ingredients as components of food, who concluded that the intended uses of D-β-hydroxybutyrate ester are safe and suitable and would be GRAS based on scientific procedures [see Appendix A, entitled “Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of (*R*)-3-Hydroxybutyl (*R*)-3-Hydroxybutyrate for Use as a Food Ingredient”]. A summary of these data is presented herein.

IV.B Regulatory Status and Dietary Sources

D-β-Hydroxybutyrate ester is GRAS in foods for special dietary purposes for the armed forces. Specifically, D-β-hydroxybutyrate ester was determined to be GRAS in specially formulated military ready-to-eat special dietary use foods (*e.g.,* nutrition bars or beverages) by U.S. military war fighters during brief periods of extreme physiological and cognitive duress (*i.e.*, under

conditions in combat). The daily dietary intake of the D-β-hydroxybutyrate ester was estimated be up to 200 g/day (equivalent to 2.9 g/kg body weight/day for a 70 kg individual), consumed in small amounts over the course of the day.

The D-β-hydroxybutyrate ester is a synthetic compound with no history of human consumption; however, its metabolites, namely (R)-1,3-butanediol and D-β-hydroxybutyrate, are reported to occur in nature. Although no quantitative data were available (levels not specified), the diol has been detected in parmesan cheese and Cupuacu, which is a commonly consumed fruit in Amazonia**,** Brazil, and Venezuela (Nijssen *et al*., 1996). In addition to its natural presence in the diet, 1,3-butanediol also is added to foods for use as a flavoring agent and solvent for flavoring agents (Burdock, 2009; FCC, 2012). Daily consumption of 1,3-butanediol as a result of its addition to foods is estimated to be approximately 0.008 mg/kg body weight/day (Burdock, 2009).

D-β-Hydroxybutyrate has been detected in cow's milk, with levels ranging from 10 to 631 µM (Larsen and Nielsen, 2005). Since ketone bodies are of mitochondrial origin, other foods of animal origin are likely to contain small amounts of D-β-hydroxybutyrate, though quantitative data were not identified in the literature.

IV.C Metabolic Fate

Like other aliphatic esters, D-β-hydroxybutyrate ester undergoes complete hydrolysis *via* carboxylesterases or esterases distributed throughout the intestinal tract, blood, liver, and other tissues (Heymann, 1980; Anders, 1989). The ketone ester is hydrolyzed to D-β-hydroxybutyrate and (R)-1,3-butanediol, with the latter being further metabolized to D-β-hydroxybutyrate and acetoacetate in the liver (Tate *et al.*, 1971; Desrochers *et al.*, 1992).

The metabolic fate of the D-β-hydroxybutyrate ester has been examined in an ascending dose study conducted in healthy volunteers (6/group) (Clarke *et al.*, 2012a). Plasma levels of D-β-hydroxybutyrate and acetoacetate were readily elevated following administration of a single drink of the ketone ester (140, 357, and 714 mg/kg body weight), while the intact compound was not detected. Maximum plasma levels of ketones were achieved within 1.5 to 2.5 hours, reaching 3.30 mM and 1.19 mM for β-hydroxybutyrate and acetoacetate, respectively, at the highest amount of the ketone ester tested. In this study, the elimination half-life was found to range from 0.77 to 3.06 hours for β-hydroxybutyrate, and from 8 to 14 hours for acetoacetate. No gender differences in the pharmacokinetic parameters of D-β-hydroxybutyrate or acetoacetate were reported.

The human pharmacokinetic study was designed to evaluate the human circulating levels of D-β-hydroxybutyrate following the administration of over double the potential maximal daily dosage (up to 2.1 g/kg/day) for 5 days. In this case the circulating D-β-hydroxybutyrate level did not exceed 5.5 mM, which is equivalent to physiological levels following a period of fasting. As

such, the human pharmacokinetic data showed that a dosage of the hydroxybutyrate ester at a value of approximately twice the estimated maximum level produced plasma levels that were considered to cause no safety concerns as they remained within the physiological normal range.

The complete and rapid hydrolysis of D-β-hydroxybutyrate ester is further supported by unpublished studies conducted in rats administered the ingredient either *via* gavage or in the diet, as well as an *in vitro* study in which D-β-hydroxybutyrate ester was incubated with fresh human plasma (data not shown).

IV.D Toxicological Studies

IV.D.1 Repeated Dose Studies

The toxicity of D-β-hydroxybutyrate ester was evaluated in a 28-day study (Clarke *et al.*, 2012b). Sixty, 9-week-old Wistar rats (10 animals per sex per group) received a diet containing D-β-hydroxybutyrate ester[1] (providing 31% of calories from D-β-hydroxybutyrate ester) or 1 of 2 control diets [a fat-based diet, providing 34% of calories from fat, or a carbohydrate (CHO)-based diet providing 70% of calories from CHOs]. The ketone ester diet was formulated to contain 30% of the calories from the D-β-hydroxybutyrate ester, resulting in a ketone ester intake of 12.0 and 15.1 g/kg body weight/day over the 28-day study for male and female rats, respectively. Animals were monitored for clinical signs, body weight, and food consumption. At the end of the study period, hematology, coagulation, clinical chemistry, and urinalysis parameters were assessed, organs were weighed, and gross and microscopic examination was undertaken.

Rats in the ketone ester group consumed significantly less diet and gained significantly less weight compared with rats in the control groups. Decreased food consumption may have been caused by the palatability of the diet containing D-β-hydroxybutyrate ester. Additionally, the reduced food consumption and weight gain in this study are consistent with reports of decreased hunger, reduced energy intakes, and increased weight loss in subjects consuming low-carbohydrate ketogenic diets compared to low-fat diets or medium-carbohydrate non-ketogenic diets (McClernon *et al.,* 2007; Johnstone *et al.*, 2008).

All animals survived to the scheduled necropsy date. Daily cage side observations and detailed weekly physical examinations showed no treatment-related toxicity in animals of both sexes in all 3 diet groups. The exception was one male rat in the ketone ester-fed group which showed piloerection, decreased food consumption, and weight loss. Piloerection was not observed after

[1] In the 28-day, 66-day, and developmental toxicity rat studies, the test material consisted of ethyl-(*R*)-3-hydroxybutyrate (~1%) and (*R*)-1,3-butanediol (~1%), which were the starting materials, and the ketone ester [~98%, representing (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate (~94%), 3-betahydroxybutryl 1,3-butanediol monoester (~1%), and di-β-hydroxybutyrate 1,3-butanediol diester (~3%)].

the third week of dosing and although this animal did not fully regain initial body weight, it was gaining weight and otherwise appeared normal by the end of the study.

Urinalysis values did not significantly differ among groups. Between-group differences were noted in several hematology, coagulation, and clinical chemistry parameters; however, mean values were within normal physiological ranges. The only exception was noted in lactate dehydrogenase (LDH) levels, which were significantly higher in ketone ester-fed rats (both sexes compared to control animals). The increases were small in magnitude and were not associated with hemolysis or histological findings. LDH levels vary greatly in rats, as demonstrated by the historical control ranges for Sprague-Dawley rats in the testing facility of 100 to 7,201 U/L in males and 20 to 5,236 U/L in females. LDH levels in the ketone ester-fed rats were within this range and thus were not considered to be of toxicological relevance. The observed elevated levels may be related to the release of LDH during regular handling of rats, such as grasping, dosing, *etc.* (Yerroum *et al*., 1999); blood collection procedures (Friedel *et al*., 1974); or enzyme release from cellular elements during clotting (Friedel and Mattenheimer, 1970). Furthermore, LDH levels were unaffected by D-β-hydroxybutyrate ester consumption in the 66-day rat study described below. Similarly, no subjects had LDH levels above the normal range in the 5-day clinical study discussed in Section IV.C.

There were no statistically significant differences in the absolute weight and in the body/brain weight ratios among groups for the following organs: spleen, liver, adrenals, testes, kidneys, prostate, lungs, heart, thymus, brain, pituitary, seminal vesicles and ovaries. The uterine weight of ketone ester-fed rats was smaller than the uterine weight of female rats fed the CHO and fat diets. No difference was noted in the relative uterus weight, indicating that the difference in absolute uterine weight was a result of the smaller body weight of female rats fed the ketone ester diet, and not an adverse effect of the ketone ester. It also should be noted that uterine weights of rats in all 3 groups were within the laboratory's historical range for uterine weight.

No gross or histopathological abnormalities of toxicological significance were observed in the heart, kidney, stomach, duodenum, ileum, colon, or brain. Two male rats and 4 female rats that received the fat diet, as well as 1 female rat given the ketone diet, had slight yellow discoloration of livers, presumed to be fat accumulation. Liver vacuolation was observed in all female rats in all 3 groups; likewise, necroinflammatory foci were observed in some animals (males and females) in all groups. Given that these findings were present in all groups and that liver function enzyme levels were within normal ranges, it is unlikely that they were related to consumption of the D-β-hydroxybutyrate ester. The diets were formulated by the separate addition of macronutrients (fat, CHO, ketone ester) to the rat chow, so the vitamin and mineral compositions of each diet were diluted, which may explain the observed findings.

With respect to muscle tissue, myocyte necrosis and repair, as well as focal histiocytosis, were noted in several animals in all 3 groups. Muscle changes were graded as minimal with the exception of 1 male and 1 female in the ketone ester group; effects in these animals were

graded as mild. There were no animals in which muscle changes were graded as moderate, marked, or severe.

Based on the results of the study, the authors concluded that that consumption of D-β-hydroxybutyrate ester at 30% of the total calorie intake (12.0 g/kg body weight/day and 15.1 g/kg body weight/day in male and female rats, respectively) in the diet did not result in adverse effects.

Another study was conducted to examine the effect of oral administration of the D-β-hydroxybutyrate ester on the physical performance and cognitive function of the rat, as well as any effect on the general health of the animals (Clarke *et al.*, unpublished). A total of 50 young male Wistar rats were procured for the study. The rats were randomized to receive one of three 1.76 kcal/g diets: 1) a Western diet (n=20), 2) a high-CHO diet (n=10) or 3) a ketone ester diet (n=20) the compositions of which are detailed in Table IV.D.1-1. After 9 days, 10 animals each from the Western diet from the ketone ester diet groups were sacrificed, while the remaining animals (10 per group) continued to receive the respective diets for a total of 66 days. The average D-β-hydroxybutyrate ester intake during the 66-day study period was 13.7 g/kg body weight/day.

Table IV.D.1-1 Composition of Test Diets

Diet	Fat (% kcal)	Protein (% kcal)	Carbohydrate (% kcal)	Ketone Ester (% kcal)
Western	34	27	39	0
Carbohydrate	4	26	70	0
Ketone	4	27	39	30

The body weights and heart weights of rats consuming the Western diet and the ketone ester diet did not differ significantly after 9 or 66 days of diet consumption. As expected, there were no corresponding differences in the ratio of body weight to heart weight. After 9 and 66 days of diet consumption the epididymal fat weight and the ratio of epididymal fat weight to body weight was significantly lower in the rats consuming the ketone ester diet than in those consuming the Western diet. These parameters also were significantly lower in the rats consuming the high-CHO diet for 66 days, compared to those consuming the Western diet. The plasma β-hydroxybutyrate levels of the rats fed the Western diet were consistently approximately 2-fold lower than those of the rats fed the ketone ester diet, 0.41 mM compared to 0.85 mM after 9 days of diet consumption and 0.48 mM compared to 0.91 mM after 66 days of diet consumption. No significant difference was observed between the levels of (R)-1,3-butanediol and acetone present in the plasma after consuming the Western and ketone ester diets for 9 days. At no time point was the D-β-hydroxybutyrate ester detected in the blood. After 9 days of consuming the experimental diets, the plasma cholesterol and triglyceride levels were significantly lower in the rats fed the ketone ester diet than those fed the Western diets. After 66 days these parameters were 52 and 40% lower, respectively, in the D-β-hydroxybutyrate

ester fed rats. The plasma glucose of the rats on the Western diet did not differ significantly from that of the rats on the ketone ester diet after 9 days; however, after 66 days the plasma glucose was 33% lower in the rats of the D-β-hydroxybutyrate ester group compared to those in the Western group. Plasma free fatty acid levels and LDH activity were not significantly different in rats consuming either diet at any time point. Additionally, no significant differences were observed in the analysis of tissues collected from Western and ketone ester-fed rats after 9 days of diet consumption.

In both the 28-day and 66-day rat studies, rats consumed D-β-hydroxybutyrate ester at levels 10 to 13 times higher than the maximum estimated intake in humans based on the proposed uses. It would have been difficult to achieve a larger margin of safety in the aforementioned studies because the ingredient is a macronutrient and will be consumed at a maximum level of 1.2 g/kg body weight/day. To attain a margin of safety of 100, the test diets in these studies would have to provide the ingredient at a level of 120 g/kg body weight/day, which would not be possible due to displacement of nutrients and the palatability of the ingredient.

IV.D.2 Reproductive and Developmental Toxicity

A developmental toxicity study was conducted wherein 25 pregnant Crl:WI(Han) rats per group were administered 2 g/kg body weight D-β-hydroxybutyrate ester or reverse osmosis deionized water (as a control substance) once daily by oral gavage on Days 6 through 20 of gestation (DGs 6 to 20), at a dosage volume of 2 mL/kg body weight (Clarke *et al.*, 2012b). On DG 21, rats were euthanized, Caesarean-sectioned, and examined for gross lesions. All fetuses were examined for external abnormalities. Approximately one-half of the fetuses in each litter were examined for visceral abnormalities, while the remaining fetuses were examined for skeletal abnormalities.

Decreased body weight gains and body weight corrected for gravid uterine weight were observed in dams administered D-β-hydroxybutyrate ester compared to controls; food consumption was similarly reduced. These findings are expected given that the test article provided caloric value whereas the control article (water) did not. Maternal alanine aminotransferase (ALT) and alkaline phosphatase (ALP) values were lower in the test group relative to controls; however, in the 28-day study, no significant effects on ALT were noted in female rats fed the ketone ester diet and ALP levels were only lower in comparison to the fat control group but not the CHO control group. Moreover, no gross or histopathological effects suggesting liver toxicity were noted.

Pregnancy occurred in 22 rats in the control group and 24 rats in test group. The litter averages for corpora lutea, implantations, the percentage of pre-implantation loss, litter sizes, live and dead fetuses, early and late resorptions, the percentage of post-implantation loss, the percentage of resorbed conceptuses, and the percentage of live male fetuses were comparable among the groups.

Male fetal body weights in the test group were significantly lower compared to controls; however, combined fetal weights did not significantly differ, the percent difference from the control group was less than 5%, and the average value was within historical ranges at the test facility.

There were no significant between-group differences in the litter or fetal incidences of any gross external, soft tissue, or skeletal abnormalities (malformations or variations), nor were there differences in fetal ossification site averages. The number of fetuses with any alteration observed and percentage of fetuses within a litter with any alteration observed were significantly higher in the D-β-hydroxybutyrate ester group compared to controls. These findings were driven by skeletal variations; however, it should be noted that the incidences of skeletal variations did not significantly differ between groups. Therefore, the observed skeletal variations were not considered to be of toxicological concern.

The authors concluded that D-β-hydroxybutyrate ester did not adversely affect the development of rats exposed to the ingredient *in utero* at a level of 2 g/kg body weight/day.

IV.E Studies in Humans

The safety and tolerability of D-β-hydroxybutyrate ester have been investigated in human studies. Ingestion of the D-β-hydroxybutyrate ester in a meal replacement milkshake beverage was without adverse effects in participants administered a single dose at up to 714 mg/kg body weight in the pharmacokinetic study described in Section IV.C. More recently, a human study was undertaken to assess the tolerability of D-β-hydroxybutyrate ester under the intended conditions of use (Clarke *et al.*, unpublished). In this randomized, blinded, placebo-controlled, cross-over study, citrus-flavored sports water drinks were used as the matrix for the ingredient as they mask the flavor of D-β-hydroxybutyrate ester more effectively compared to milkshake. Forty-two adult men consumed a vitamin water drink to which was added 1.23 g β-hydroxybutyrate ester/kg body weight or 1.44 g dextrose/kg body weight (both drinks also contained 0.1 g fructose/mL). The subjects drank a total volume of 7.6 mL/kg body weight (approximately 578 mL total volume), divided into 3 drinks of equal volume, at 10 min before starting the exercise session, and at 65 min and 130 min of the cycling session (so 1.23 g β-hydroxybutyrate ester/kg body weight consumed within 140 minutes). There was a 72-hour washout period between each test protocol.

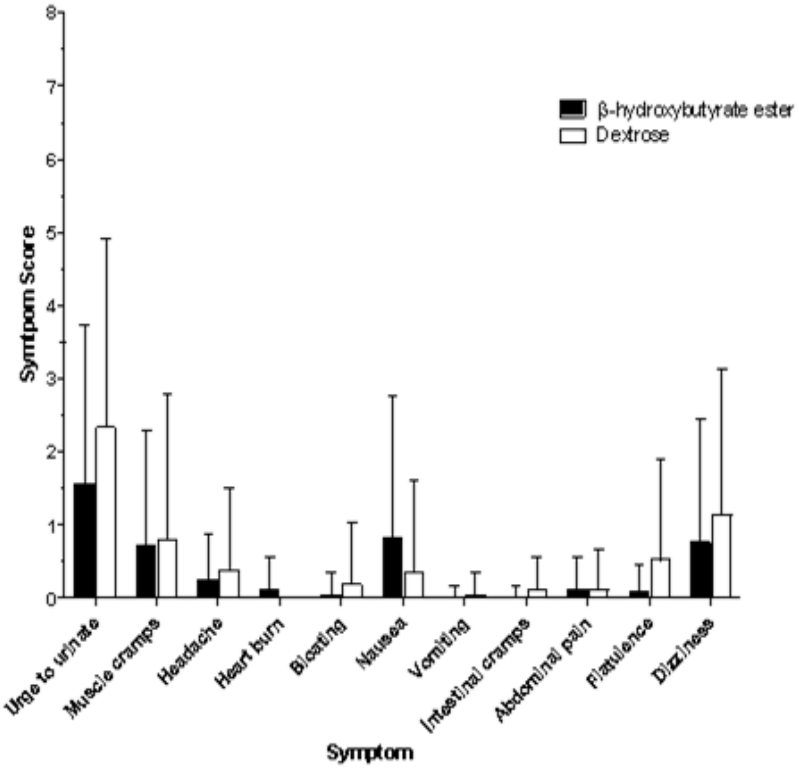
Subjects completed a Gastrointestinal Symptoms Questionnaire 7 times during the protocol, at 0, 40, 65, 105, 130, 170, and 195 minutes of the exercise session. In the questionnaire, subjects rated the severity of symptoms on a scale of 0 (none) to 8 (unbearable) for upper abdominal problems (heartburn, bloating, nausea, and vomiting), lower abdominal problems (intestinal cramps, abdominal pain, flatulence, and diarrhea), and systemic problems (dizziness, headache, muscle cramp, and urge to urinate). The most severe score for each symptom was chosen as the final score for each subject. A score of 0 was classified as “none”, scores of 1, 2, or 3, were classified as “mild”, scores of 4 or 5 were classified as “moderate”, scores of 6 or 7

were classified as “severe”, and a score of 8 was classified as “unbearable”. Blood samples were collected concurrently with the completion of the questionnaire for the measurement of D-β-hydroxybutyrate, glucose, lactate, glycerol, insulin, and free fatty acid levels.

Blood D-β-hydroxybutyrate levels increased to ~1 mM 10 min after the first drink of ketone monoester, rising with each successive drink to reach ~4.0 mM at the end of exercise. Levels of glucose, lactate, glycerol, insulin, and free fatty acids in the blood were not adversely impacted by the consumption of D-β-hydroxybutyrate ester.

As shown in Figure IV.E-1, gastrointestinal symptoms occurred in few subjects, and there were no significant differences in symptom severity between the D-β-hydroxybutyrate ester and placebo arms of the study. No severe adverse events occurred. The findings of this study demonstrate that D-β-hydroxybutyrate ester is well-tolerated under the intended conditions of use (*i.e.*, provided at a dose consistent with the proposed uses or the ingredient, consumed before and during exercise, and consumed in reasonable volumes in a sports drink matrix).

Figure IV.E-1 Gastrointestinal Symptoms in Physical Endurance Study



A study in which healthy volunteers drank only the D-β-hydroxybutyrate ester in a milkshake drink for 5 days also has been conducted (Clarke *et al.*, 2012a). Participants (6/sex/group) consumed a meal replacement milkshake beverage containing 140, 357, or 714 mg/kg body

weight of the ketone ester 3 times daily, resulting in daily intakes of 420, 1,071, and 2,142 mg/kg body weight/day. Each 100 g serving of the meal replacement milkshake contained approximately 84 kcal and 5.2 g of the ketone ester. To standardize total daily caloric intake to 34 kcal/kg body weight, subjects in the low-dose (420 mg/kg body weight/day) and mid-dose (1,071 mg/kg body weight/day) groups received Ensure®, a water-based beverage, as a supplemental formula. Subjects receiving the lower doses of β-hydroxybutyrate ester consumed a lower total amount of liquid (ketone monoester-containing milkshake + Ensure®) than those who received the high dose of β-hydroxybutyrate ester (Table IV.E-1). It should be noted that the highest daily dose administered in this study (2,142 mg/kg body weight) is beyond the maximum estimated daily intake of D-β-hydroxybutyrate ester based on the proposed uses (1,200 mg/kg body weight) and that the matrix in which the ingredient was delivered (milkshake) will not be utilized under the intended conditions of use. Nevertheless, the results of this study are included for the purposes of completeness.

Table IV.E-1 Amount of D-β-Hydroxybutyrate Ester-Containing Milkshake and Ensure Consumed per Occasion in 5-Day Human Study (Clarke *et al.*, 2012a)

Dose of ketone monoester (mg/kg body weight)	Amount of ketone drink consumed (g/kg body weight)	Mean body weight (kg)	Amount of ketone drink consumed (g)	Amount of Ensure consumed (g/kg body weight)	Amount of Ensure consumed (g)	Total amount (ketone drink + Ensure) consumed (g)
140	2.69	76.1	205	8.72	664	868
357	6.87	73.8	507	5.35	395	902
714	13.73	77.7	1,066	0	0	1,066

Ketone and glucose levels were carefully monitored to ensure that β-hydroxybutyrate levels remained within the normal range (0 to 5.5 mM) and that hypoglycemia or hyperglycemia did not develop over the course of the study.

No abnormal changes in the levels of blood lipids, as well as hematology, clinical biochemistry, or urinalysis were observed following all doses in both the single and repeated dose study. Moreover, blood ketone levels and glucose levels did not deviate from ranges deemed to be safe, with blood D-β-hydroxybutyrate not exceeding 5.5 mM, and glucose levels remaining above 65 mg/dL (2.5 mM) and below 400 mg/dL (22.2 mM). Blood ketone levels increased in all subjects following milkshake consumption, indicating that the test substance was absorbed. Vital signs also were stable throughout the course of the study, and no ketone-related abnormalities were reported upon physical examination for all participants.

Adverse events that were deemed possibly to be treatment-related were observed in 4 out of 12 participants in the low-dose group and 1 out of 12 participants in the mid-dose group. The low and mid doses of β-hydroxybutyrate ester were generally well tolerated, and the few adverse events reported were all considered to be mild and only “possibly” related to β-hydroxybutyrate ester consumption. Mild gastrointestinal symptoms were observed in only 2 individuals receiving

the low dose (420 mg/kg body weight/day; 1 incidence of nausea in 1 female and 1 incidence of flatulence in 1 male), and only 1 individual receiving the mid-dose (1,071 mg/kg body weight/day; 1 male with 1 incidence of diarrhea and decreased appetite).

At the highest dose administered (2,142 mg/kg body weight/day), although adverse events were reported, in most instances the effects were considered to be mild and occurred only once in each subject during the 5-day dosing period. Most of the adverse events reported were gastrointestinal in nature (flatulence, nausea, diarrhea, constipation, vomiting, and abdominal distension and pain), some of which were deemed to be "highly probable" in their relation to D-β-hydroxybutyrate ester consumption, being attributed to the consumption of a large amount of a thick milk-based beverage (1,066 g in the high-dose group) over a short duration. Subjects were initially asked to consume the drink (1.1 liters) within 1 minute on each occasion, though this time period was extended to 30 minutes when some of the individuals vomited immediately following consumption. Additionally, the taste of D-β-hydroxybutyrate ester, which is bitter and sharp, was likely also a contributing factor to the reported nausea and vomiting.

The reported gastrointestinal effects occurred at a lower frequency in individuals who consumed lower doses of D-β-hydroxybutyrate ester because they received a smaller volume of the test substance-containing milkshake as shown in Table IV.E-1. Consumption of Ensure®, a water-based beverage, would not be expected to elicit the same gastrointestinal effects as an equal volume of milkshake.

As mentioned, most of the reported gastrointestinal effects were mild and only occurred once during the study. Repeated occurrences of a gastrointestinal effect in the same individual were reported only in 15 instances (*i.e.*, in 13 instances, the reported effect occurred twice in the same individual, and in 2 instances, the reported effect occurred 3 times in the same individual). The effects did not progress in severity, with the exception of vomiting, which progressed from moderate to severe in 1 individual, who was subsequently discontinued from the study because of the vomiting. Since vomiting occurred immediately after consumption of the milkshake, this effect may be attributed to the taste of D-β-hydroxybutyrate ester, as well as the large volume consumed within a short duration, rather than to a toxicological effect of the test substance. A second subject in the high-dose group was discontinued from the study due to reports of several adverse effects, of which the gastrointestinal effects and chest pain were considered to be "highly probable" or "probable" in their relation to the treatment. These effects may be attributed to the large volume of milkshake consumed over a short period of time.

In addition to the gastrointestinal effects, headaches, dizziness, lethargy, and somnolence were reported in some participants, although these were considered to be mild in severity and were deemed to be "probable" in relation to D-β-hydroxybutyrate ester treatment. All other adverse events reported were considered mild in severity with "probable" or "possible" relation to ketone ester consumption. The adverse events reported at all doses of ketone ester resolved

spontaneously by the end of the study, with the exception of the positive fecal occult test observed in one individual in the lowest dose group.

The reported adverse events in the 5-day human study are indicative of a physiological response to the food matrix (milkshake) and dosing regimen (consumption of ~1.1 L within 1 minute), rather than a toxicological effect of β-hydroxybutyrate ester *per se*. The generally mild and isolated nature of the reported adverse events helps to support this conclusion.

In summary, results of the physical endurance study (Clarke *et al.*, unpublished) support the safety of D-β-hydroxybutyrate ester under the proposed conditions of use (provided at a total daily amount of 1.23 g/kg body weight, consumed before, during and/or following exercise, and consumed in reasonable volumes of less than 200 mL in a sports drink matrix). While gastrointestinal effects were noted in the high-dose group (2,142 mg/kg body weight/day) from the study reported by Clarke *et al.* (2012a), these effects may be attributed to the study design [*i.e.*, matrix, volume, and directions for use; see Table IV.E-2 for a comparison of the 2 clinical studies]. Additionally, only 2 mild adverse effects were reported in the mid-dose group (1,071 mg/kg body weight/day), who consumed D-β-hydroxybutyrate ester at levels consistent with the intended uses of the ingredient. As such, findings of the Clarke *et al.* (2012a) study provide corroborative evidence of safety.

Table IV.E-2 Comparison of Human Studies Conducted on β-Hydroxybutyrate Ester

Reference	Food Matrix	Instructions for Use	Dose of β-hydroxybutyrate ester per occasion (mg/kg body weight)	Daily dose of β-hydroxybutyrate ester (mg/kg body weight) 3 drinks/day	Volume per drink (mL)*	Daily Volume (mL)*
Clarke *et al.*, unpublished	Vitamin water drink	Subjects asked to consume the drink within 5 to 10 minutes	410	1,230	193	578
Clarke *et al.*, 2012a	Milkshake	Subjects initially asked to consume the drink within 1 minute on each occasion; time period extended to 30 minutes	140 357 714	420 1,071 2,142	868 902 1,066	2,605 2,706 3,200

*Amount of drink consumed was reported in grams by Clarke *et al*. (2012a); for the purposes of comparing studies, 1 g was considered equivalent to 1 mL volume.

IV.F Additional Considerations

IV.F.1 Studies Pertaining to the Safety of the Metabolites of D-β-Hydroxybutyrate Ester

The safety of D-β-hydroxybutyrate ester is supported by the results of studies conducted on its metabolites, D-β-hydroxybutyrate and (R)-1,3-butanediol. Sodium D,L-β-hydroxybutyrate has been administered orally to children with acyl-CoA dehydrogenase deficiency or with persistent hyperinsulinemic hypoglycemia at dose levels up to 1,000 mg/kg body weight/day with no side effects (Plecko *et al.,* 2002; Van Hove *et al.,* 2003). The results of *in vitro* studies (with isolated embryos) suggest that physiologically relevant levels of β-hydroxybutyrate, particularly the L-isomer, may disrupt normal embryogenesis (Sheehan *et al.,* 1985; Hunter *et al.,* 1987; Moley *et al.,* 1994); however, the results of an *in vivo* study with R,S-1,3-butanediol indicate that it is not teratogenic (Hess *et al.,* 1981). Because 100% and 30% of the R and S enantiomers, respectively, of 1,3-butanediol are metabolized to ketones (Desrochers *et al.*, 1992), studies on 1,3-butanediol indirectly provide information on the reproductive and developmental safety of β-hydroxybutyrate. Moreover, results of the rat developmental toxicity study on D-β-hydroxybutyrate ester indicated no adverse effects on development.

Almost all experimental studies on 1,3-butanediol identified administered both the (R)- and (S)-forms of butanediol, except for the chronic toxicity studies conducted by Scala and Paynter (1967). In this study, it was demonstrated that the 2-year oral administration of (R)-1,3-butanediol to Sprague-Dawley rats or to pure bred-beagles, at levels of 5 and 0.8 g/kg body weight/day, respectively, was not associated with toxicity. Hess *et al.* (1981) demonstrated, in 5 successive breedings of Wistar rats, that consumption of 5 to 24 g/kg body weight/day of 1,3-butanediol was not associated with developmental effects. The control and test groups were comparable with respect to gestation, viability, and lactation indices. The Acceptable Daily Intake established by JECFA for 1,3-butanediol is 4 mg/kg body weight/day (JECFA, 1980). Short-term human experimental studies indicate that 1,3-butanediol consumption may result in statistically, but not clinically significant reductions in blood glucose levels (Kies *et al.*, 1973; JECFA, 1980); however, it should be noted that no clinically significant effects on blood glucose levels were reported in the 5-day human study in which subjects consumed up to 2 g D-β-hydroxybutyrate ester/kg body weight/day (Clarke *et al.*, 2012a).

IV.F.2 Studies Conducted with 1,3-Butanediol Diacetoacetate

The safety of D-β-hydroxybutyrate ester is further supported by animal feeding trials of a similar ketone ester, namely (R,S)-1,3-butanediol mono- and diacetoacetate. A bolus intragastric dose of approximately 1.3 g/kg body weight administered to pigs was not associated with alterations in standard clinical chemistry parameters or with deleterious side effects (Desrochers *et al.,* 1995). Likewise, the administration of repeated oral doses of (R,S)-1,3-butanediol diacetoacetate over a 300-minute period (equivalent to 1,054 to 1,144 mg/kg body weight) or a single bolus dose of 439 to 477 mg/kg body weight (R,S)-1,3-butanediol diacetoacetate to dogs

was not associated with alterations in clinical chemistry analyses; moreover, the authors reported no signs of distress in any of the animals during or following the experiment (Puchowicz *et al.,* 2000).

IV.F.3 Endogenous Production of Ketones

Humans have evolved to endure specific adverse conditions, such as going without food, for significant periods of time. During food deprivation, the body's natural homeostatic mechanism is to obtain energy from ketone bodies to lower glucose utilisation and muscle protein breakdown. As a result, circulating ketone bodies rise to fuel the body's need for energy to levels (2 to 3 mM), similar to those of high-performance athletes consuming the β-hydroxybutyrate ester drink. Raising the levels of circulating ketone bodies is therefore a natural mechanism that is used for human survival and shows clearly that elevated circulating ketones protect the body during adverse conditions or dietary manipulation. Since the development of *Homo sapiens* approximately 100,000 years ago, prolonged fasting has been the normal state of the species. As the brain in *Homo sapiens* grew in size, in comparison to failed earlier species *Homo robustus* and *habilis,* the larger brain required metabolic adaptations to meet its increased energy requirements during periods of scarcity. During the 95,000 years *Homo sapiens* existed as hunter-gatherers, long periods of food scarcity and famine were the rule, so the ability to survive required ketosis. The 1.5 kg brain, which under fed conditions utilizes only glucose and requires 20% of total oxygen consumed, has been estimated to survive only 6 days if the sole source of fuel is glucose made from amino acids. Instead, the evolution of ketosis allowed a normal weight male to survive approximately 65 to 75 days. In this evolutionary light, ketone bodies should be considered a fourth class of normal nutrient, particularly for humans, joining proteins, fats and carbohydrates. Ketone bodies differ only from the other 3 great nutrient classes in that they are generated internally from mobilized fat, rather than consumed. Thus, ketone bodies are an essential nutrient required for human survival throughout history.

Studies by Cahill (1970 and 2006) showed that brain utilizes ketone bodies during starvation with no untoward effects of ketosis either on physiological state or cognition. The relationship between ketosis and brain function was first made clear by the classic studies of George Cahill of fasting-induced ketosis in man. After approximately 5 days of fasting, total blood ketone bodies is elevated to between 5-7 mM (Robinson and Williamson, 1980). At that level, approximately 60% of the metabolic demand of brain of fasting man was supplied by ketone bodies (Cahill, 1970). Most interestingly, hunger disappeared after about the third day of fasting and there were no observed toxic effects of this prolonged ketotic state. In another experiment on ketotic fasting subjects, Cahill, using insulin, lowered blood sugar to 1 mM and observed neither seizures nor any obvious impairment of cognitive function at levels of blood sugar which, in the absence of ketone bodies, would produce both.

During fasting or states of low insulin, free fatty acids are released from adipocytes where they are transported to liver and converted by β-oxidation to acetyl CoA. Ketone bodies are formed in the mitochondria from acetyl CoA by the following reactions:

$$2\,\text{acetyl CoA} \xleftrightarrow{\text{Thiolase}} \text{acetoacetyl CoA} + \text{acetyl CoA} \xrightarrow{\text{HMGCoA Synthase}} \text{HMG CoA} + \text{CoA}$$

$$\xrightarrow{\text{HMGCoA Lyase}} \text{Acetoacetate} + \text{Acetyl CoA}$$

HMG CoA is also formed in the cytosol where it is converted to mevalonate and hence to cholesterol, a process independent of mitochondrial ketone body synthesis. In fasting man, about 150 g of ketone bodies are synthesized per day (Robinson and Williamson, 1980). To increase total blood ketone levels in a non exercising human, one would have to feed approximately this amount. In exercising humans, this amount would increase in proportion to energy demand (Balasse *et al.*, 1978; Balasse and Féry 1989; Féry and Balasse 1983, 1986).

The rate of ketone formation is low when an adequate supply of carbohydrates is available; however, when blood glucose levels decrease, the rate of ketogenesis is increased, and ketones replace glucose as the primary source of energy. The normal 6- to 8-hour fasting blood ketone concentration of a healthy individual is reported to be approximately 0.5 mM, while after a 5- to 7-day fast, total blood ketone levels as high as 5 to 7 mM have been documented (Owen *et al*., 1967; Mensink *et al.,* 1992; VanItallie and Nufert, 2003). Typical levels of β-hydroxybutyrate in the blood are reported to be approximately 0.2 mM, with levels increasing by up to 50 times during periods of limited calorie intake (Hall *et al*., 1984). The normal blood ketone level of pregnant women is slightly higher than that of other individuals, and pregnant women, because of their greater caloric requirements, achieve greater ketone levels during times of limited glucose availability (Paterson *et al.,* 1967; Gin *et al.*, 2006). In late pregnancy, blood ketone levels are reported to reach 4 to 6 mM after 48 hours of fasting (Robinson and Williamson, 1980). Levels after prolonged fasting and in late pregnancy are consistent with the maximum blood D-β-hydroxybutyrate levels reached (~4.0 mM) following consumption of the ketone ester under the intended conditions of use (Clarke *et al.*, unpublished). Maximal ketone body production by a healthy adult liver and kidney is approximately 185 g/day, with ketones accounting for between 2 and 6% of an individual's energy needs after an overnight fast and up to 40% of energy needs after a 3-day fast (Reichard *et al.*, 1974; Laffel, 1999).

In most mammals including humans, the fatty acid oxidation product, acetyl-CoA, formed in the liver can enter the citric acid cycle or alternatively can be converted to ketone bodies, a metabolic process called ketogenesis (Nelson and Cox, 2000). Ketogenesis occurs mainly in the mitochondria of liver cells during times of limited glucose availability, when there is an increase in lipolysis and a concomitant reduction or saturation in acetyl-CoA oxidation in the citric acid cycle. The latter occurs as a result of the need for citric acid cycle intermediates to enter the gluconeogenic pathway to maintain blood glucose levels. Acetyl-CoA, the primary substrate in the citric acid cycle, is then diverted into the ketogenic pathway, resulting in the

formation of the ketone bodies, acetoacetate and D-β-hydroxybutyrate. Acetoacetate and D-β-hydroxybutyrate are transported to extra-hepatic tissues, where they can be oxidized in the citric acid cycle and thus serve as an alternate source of energy. D-β-hydroxybutyrate is oxidized to acetoacetate by D-β-hydroxybutyrate dehydrogenase, which is subsequently converted to acetoacetyl-CoA and finally to 2 acetyl-CoA molecules. While D-β-hydroxybutyrate dehydrogenase is found in the liver, 3-oxoacid-CoA transferase, which converts acetoacetate to acetoacetyl-CoA, does not occur in hepatic tissue. Thus, the liver cannot utilize ketone bodies as an alternate source of energy. In the fed state, the brain uses glucose as its primary source of energy; however, the brain also can use acetoacetate and D-β-hydroxybutyrate when these metabolites are available. Production of ketone bodies, and their transport to other tissues for conversion to acetyl-CoA, allows for continued oxidation of fatty acids, particularly when acetyl-CoA oxidation slows down. A reduction in acetyl-CoA oxidation may occur when intermediates of the citric acid cycle are being used in gluconeogenesis or during coenzyme A saturation.

The oral administration of the D-β-hydroxybutyrate ester can be considered a direct means of increasing systemic ketone levels, thereby providing additional acetyl-CoA substrates for the citric acid cycle.

IV.F.4 Ketogenic Diets

Ketogenic diets are high-fat, adequate protein, low-carbohydrate diets that induce and maintain ketosis in the body. The “classic” ketogenic diet, which consists of fats and carbohydrates in a 4:1 ratio, was developed in 1921 for use for the treatment of pediatric refractory epilepsy (Wilder, 1921), and continues to be utilized as an anticonvulsant dietary regimen.

The standard Mayo “ketogenic diet” is rarely used in patients over 17 years of age because of elevation of serum cholesterol and consequent atherogenic risk (Keene, 2006; Kossoff and Rho, 2009). More recently, a “hyper-ketogenic diet” comprised of unsaturated fats has been developed where blood ketone levels achieved are higher, in the 5-8 mM range, and elevation of serum cholesterol much less marked. There are however, difficulties with patient compliance in this mainly lipid diet (Keene, 2006). The information above was used as the basis for developing the D-β-hydroxybutyrate ester scientific program that underpins the GRAS determination.

People consuming a ketogenic (Atkins) diet have high levels of circulating ketones. Such ketogenic diets which have been in existence for around 100 hundreds of years indicate no significant adverse effects have resulted from elevated plasma ketone concentrations. These examples provide substantial evidence, from an historical perspective, that high ketone body levels do not result in significant adverse health effects over the long-term and, consequently, these findings are of significant relevance to understanding the general safety of the ketone ester.

In addition to their established role in the treatment of pediatric epilepsy, ketogenic diets have potential therapeutic applications in neurodegenerative diseases, including Alzheimer's (Reger *et al.*, 2004) and Parkinson's disease (Vanltallie *et al.*, 2005). Variations of the ketogenic diet also have been investigated for their therapeutic effects on obesity (Dashti *et al.*, 2003, 2006; Yancy *et al.*, 2004), diabetes (Westman *et al.*, 2008; Al-Khalifa *et al.*, 2009), cancer (Freedland *et al.*, 2008; Otto *et al.*, 2008; Seyfried *et al.*, 2008), and a number of other conditions.

Similar to ketogenic diets, D-β-hydroxybutyrate ester will be used to elevate blood ketone levels, given that it is ultimately metabolized to the ketones D-β-hydroxybutyrate and acetoacetate following ingestion. The long history of use of ketogenic diets provides corroborative evidence of D-β-hydroxybutyrate ester's safety for its intended use.

IV.G Summary and Basis for GRAS Conclusion

The GRAS determination for the use of D-β-hydroxybutyrate ester as a food ingredient is based on scientific procedures. D-β-Hydroxybutyrate ester has been developed as an oral source of ketones, which will be utilized as an energy source for high-performance athletes and persons undergoing strenuous exercise or conditions leading to rapid energy depletion. TΔS intends to use D-β-hydroxybutyrate ester in beverages, bars, and gels. Considering that D-β-hydroxybutyrate ester-containing products will be consumed only by high-performance athletes and persons undergoing extreme energy expenditures, the food products will be consumed during training and competition to support performance. The ingredient will not be used in mainstream foods. It is therefore likely that D-β-hydroxybutyrate ester-containing products will be used intermittently and by a small section of the population. The addition of D-β-hydroxybutyrate ester to the proposed products will result in intakes that will not exceed 1.2 g/kg body weight/day. Based on an average 70 kg adult, this maximum will relate to 75 g D-β-hydroxybutyrate ester per day. It is envisaged that a maximum of 2 to 3 servings per day will be consumed depending on the strenuous exercise taking place such as a triathlon or long haul cycle race.

D-β-Hydroxybutyrate ester is produced *via* an enzyme-catalyzed transesterification reaction of ethyl-D-hydroxybutyrate and (R)-1,3-butanediol, using a lipase preparation from *Aspergillus niger* containing the lipase gene from *Candida antarctica*. No other raw materials or processing aids are utilized. The final product contains ≥97.5% D-β-hydroxybutyrate ester. Recent batch analyses for 3 lots of D-β-hydroxybutyrate ester demonstrate compliance with product specifications and production of consistent products.

Following oral administration, D-β-hydroxybutyrate ester is fully hydrolyzed to D-β-hydroxybutyrate and (R)-1,3-butanediol in the gut. The latter is further metabolized to D-β-hydroxybutyrate and acetoacetate in the liver. The available data suggest ready bioavailability and rapid elimination of the metabolites.

The safety of D-β-hydroxybutyrate ester is supported by the results of 28-day toxicity study conducted in rats in which the consumption of diets containing 11.4% D-β-hydroxybutyrate ester (12 and 15 g/kg body weight/day in male and female rats, respectively) did not cause toxicological effects. Rats in the ketone ester group consumed significantly less feed and gained significantly less weight than rats in the control groups. Decreased food consumption may have resulted from the palatability of the diet containing D-β-hydroxybutyrate ester. Additionally, these effects are consistent with reports of decreased hunger, reduced energy intakes, and increased weight loss in subjects consuming low-carbohydrate ketogenic diets compared to low-fat diets or medium-carbohydrate non-ketogenic diets. Clinical chemistry analysis revealed that lactate dehydrogenase levels were significantly higher in ketone ester-fed rats (both sexes compared to control animals); however, the increases were small in magnitude and were not associated with changes in hemolysis parameters or histopathological effects. Microscopic findings (*e.g.*, vacuolation) in the liver were present in all groups and were not accompanied by effects on liver function parameters; therefore, they were not considered to be related to consumption of D-β-hydroxybutyrate ester.

Results from a developmental toxicity study in rats also support the safety of D-β-hydroxybutyrate ester. Administration of 2 g D-β-hydroxybutyrate ester/kg body weight/day *via* gavage on Gestation Days 6 through 20 did not affect reproductive performance or litter parameters. Litter averages for corpora lutea, implantations, the percentage of pre-implantation loss, litter sizes, live and dead fetuses, early and late resorptions, the percentage of resorbed conceptuses, and the percentage of live male fetuses were comparable among groups. The overall incidence of fetal alterations (gross external, soft tissue, and skeletal combined) was significantly higher in the ketone ester group; however, there were no significant between-group differences in the litter or fetal incidences of any gross external, soft tissue, or skeletal abnormalities (malformations or variations) when assessed individually.

In a physical endurance study in which adult men consumed a vitamin water drink containing 1.23 g D-β-hydroxybutyrate ester/kg body weight or 1.44 g dextrose/kg body weight (divided into 3 drinks of equal volume) before and during a cycling exercise session, D-β-hydroxybutyrate ester was well tolerated under conditions similar to the intended conditions of use, with no significant differences in symptom severity between the D-β-hydroxybutyrate ester and control arms of the study. In another human study conducted in healthy male and female subjects, D-β-hydroxybutyrate ester was administered in a milkshake matrix at doses of 140, 357, and 714 mg/kg body weight 3 times daily (equivalent to 420, 1,071, and 2,142 mg/kg body weight/day) over a period of 5 days. The highest amount tested being equivalent to twice the recommended daily intake. No abnormal changes in hematology, clinical biochemistry, or urinalysis parameters were observed and levels of blood lipids, ketones, or glucose did not deviate from normal physiological ranges that occur during fasting or through the use of a ketogenic diet. The ketone ester was generally well tolerated, although some gastrointestinal effects were reported at the highest dose tested. In most instances, the effects were considered

to be mild and occurred only once in each subject during the 5-day dosing period. These effects were attributed to the food matrix (milkshake) and dosing regimen (consumption of ~1.1 L within 1 minute) rather than to a toxicological effect of the test substance. Additionally, the taste of D-β-hydroxybutyrate ester, which is bitter and sharp, was likely a contributing factor. The reported gastrointestinal effects occurred at a lower frequency in individuals who consumed lower doses of D-β-hydroxybutyrate ester because they received a smaller volume of the test substance-containing milkshake.

The safety of D-β-hydroxybutyrate ester for its intended use is corroborated by results of studies conducted on its metabolites, D-β-hydroxybutyrate and (R)-1,3-butanediol. Data pertaining to a similar ketone ester, namely (R,S)-1,3-butanediol mono- and diacetoacetate, also support safety.

As discussed, the metabolites of D-β-hydroxybutyrate ester are ketones, which are produced in the body as a source of energy, particularly when blood glucose levels decrease. Blood ketone levels following prolonged fasting in healthy individuals, as well as in late pregnancy, are consistent with the maximum blood D-β-hydroxybutyrate levels reached (~4.0 mM) following consumption of the ketone ester under the intended conditions of use. Additionally, maximal ketone body production is approximately 185 g/day during times of limited glucose availability such as fasting, indicating that the human body has the capacity to handle large amounts of ketones and further corroborating the safety of D-β-hydroxybutyrate ester for the general population. Moreover, there is a long history of use of ketogenic diets, which have been used for nearly a century in the treatment of pediatric refractory epilepsy. Ketogenic diets elevate ketone levels, similar to D-β-hydroxybutyrate ester.

Together, the information provided above support the conclusion that the consumption of D-β-hydroxybutyrate ester under the intended conditions of use would not be expected to produce adverse effects in consumers. Food products containing the ingredient will be specifically targeted to high performance athletes during exercise, but the available information supports the safety of D-β-hydroxybutyrate ester for consumption at the proposed levels of use by the general population.

Overall, the D-β-hydroxybutyrate ester safety evaluation program was developed on the understanding that D-β-hydroxybutyrate is an endogenously produced compound that provides unique energy qualities that increase in concentration during periods of fasting or diet manipulation. Since increases in the endogenous production of ketone bodies are considered a natural homeostatic mechanism required to support the energy requirements of the brain, these normal physiological processes were determined to underpin the safety of the ester form. Animal and human metabolism studies clearly show, as would be expected, the ester is cleaved by esterases primarily in the gut to yield the naturally circulating D-β-hydroxybutyrate and that ester dosage levels greater than 2-fold higher than the recommended daily dosage produce plasma levels within physiological ranges, which support the safety of the material.

Toxicological safety studies were conducted at high dietary concentrations that provide a 10-fold margin of safety to those dose concentrations known to produce performance benefits within elite athletes (75 g). Overall, no adverse findings have been reported within cell, animal and human safety studies at high dose levels with the D-β-hydroxybutyrate ester. The fact that the ester is metabolized to the naturally circulating ketone body to produce physiological plasma levels supports the safety of the ingredient at the anticipated usage level.

Finally, the Expert Panel convened on behalf of TΔS, independently and collectively, critically evaluated the data and information summarized above and concluded that the intended uses of D-β-hydroxybutyrate ester, produced in accordance with cGMP and meeting appropriate food-grade specifications, are safe and suitable. Furthermore, the Expert Panel unanimously concluded that the intended uses of D-β-hydroxybutyrate ester are GRAS based on scientific procedures. It is also the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, TΔS has concluded that D-β-hydroxybutyrate ester is GRAS under the intended conditions of use on the basis of scientific procedures. As such, the ingredient is excluded from the definition of a food additive and thus may be marketed and sold for the uses designated above in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

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Appendix A

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of (*R*)-3-Hydroxybutyl (*R*)-3-Hydroxybutyrate for Use as a Food Ingredient

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of (*R*)-3-Hydroxybutyl (*R*)-3-Hydroxybutyrate for Use as a Food Ingredient

July 25, 2013

INTRODUCTION

TΔS Limited (TΔS) convened a panel (the "Expert Panel") of independent scientists, qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, to conduct a critical and comprehensive evaluation of the available pertinent data and information on (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate, referred to as D-β-hydroxybutyrate ester hereafter, and to determine whether the proposed uses of D-β-hydroxybutyrate ester as food ingredients, would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Panel consisted of the below-signed qualified scientific experts: Dr. Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Dr. Robert J. Nicolosi (University of Massachusetts Lowell), and Dr. John A. Thomas (Indiana University School of Medicine).

The Expert Panel, independently and collectively, critically examined a comprehensive package of scientific information and data compiled from the literature and other published sources including opinions from regulatory authorities and scientific bodies. This information was presented in a dossier [Documentation Supporting the Evaluation of (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate as Generally Recognized as Safe (GRAS) for Use as a Food Ingredient] that was submitted by TΔS to the Expert Panel. In addition, the Expert Panel evaluated other information deemed appropriate or necessary, including data and information provided by TΔS. The data evaluated by the Expert Panel included information pertaining to the method of manufacture and product specifications, analytical data, intended use levels, consumption estimates, and a comprehensive assessment of the available scientific literature pertaining to the safety of D-β-hydroxybutyrate ester.

Following independent, critical evaluation of such data and information, the Expert Panel unanimously concluded that the intended uses described herein of D-β-hydroxybutyrate ester, meeting appropriate food-grade specifications, and manufactured consistent with current Good Manufacturing Practice (cGMP), are GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion appears below.

SUMMARY AND BASIS FOR GRAS

TΔS intends to market D-β-hydroxybutyrate ester for use as a food ingredient in the United States (U.S.). D-β-Hydroxybutyrate ester has been developed as an oral source of ketones, which will be utilized as an energy source for high-performance athletes and persons undergoing strenuous exercise or conditions leading to rapid energy depletion.

D-β-Hydroxybutyrate ester is produced *via* an enzyme-catalyzed transesterification reaction of ethyl-D-hydroxybutyrate and (R)-1,3-butanediol, using a lipase preparation from *Aspergillus niger* containing the lipase gene from *Candida antarctica*. No other raw materials or processing aids are utilized. The final product contains ≥97.5% D-β-hydroxybutyrate ester. Recent batch analyses for 3 lots of D-β-hydroxybutyrate ester demonstrate compliance with product specifications and production of consistent products.

D-β-Hydroxybutyrate ester was previously determined to be GRAS in formulated Military Ready-to-Eat special dietary use foods (*e.g.,* nutrition bars or beverages) by U.S. military war fighters during brief periods of extreme physiological and cognitive duress (*i.e*., under conditions in combat). In the current GRAS determination, TΔS intends to use D-β-hydroxybutyrate ester in beverages, bars, and gels. Considering that D-β-hydroxybutyrate ester-containing products will be consumed only by high-performance athletes and persons undergoing extreme energy expenditures, the food products will be consumed during training and competition to support performance. The ingredient will not be used in mainstream foods. It is therefore likely that D-β-hydroxybutyrate ester-containing products will be used intermittently and by a small section of the population. The addition of D-β-hydroxybutyrate ester to the proposed products will result in intakes that will not exceed 1.2 g/kg body weight/day. Based on an average 70 kg adult, this maximum will relate to 75 g D-β-hydroxybutyrate ester per day. It is envisaged that a maximum of 2 to 3 servings per day will be consumed. These products will be targeted to high-performance athletes only.

Like other aliphatic esters, D-β-hydroxybutyrate ester undergoes complete hydrolysis *via* carboxylesterases or esterases distributed throughout the intestinal tract, blood, liver, and other tissues (Heymann, 1980; Anders, 1989). The ketone ester is hydrolyzed to D-β-hydroxybutyrate and (R)-1,3-butanediol, with the latter being further metabolized to D-β-hydroxybutyrate and acetoacetate in the liver (Tate *et al.*, 1971; Desrochers *et al.*, 1992). In a human pharmacokinetic study, plasma levels of D-β-hydroxybutyrate and acetoacetate were readily elevated, reaching peak levels of 3.30 and 1.19 mM, respectively, within 1.5 to 2.5 hours following administration of a single dose of the ketone ester (up to 714 mg/kg body weight), while the intact compound was not detected (Clarke *et al.*, 2012a). The elimination half-life ranged from 0.77 to 3.06 hours for β-hydroxybutyrate, and from 8 to 14 hours for acetoacetate. These data suggest ready bioavailability and rapid elimination.

A 28-day toxicity study conducted in rats demonstrated that the consumption of diets containing 11.4% D-β-hydroxybutyrate ester (12 and 15 g/kg body weight/day in male and female rats, respectively) did not cause toxicological effects (Clarke *et al.*, 2012b). Rats in the ketone ester group consumed significantly less feed and gained significantly less weight than rats in the control groups. Decreased food consumption may have resulted from the palatability of the diet containing D-β-hydroxybutyrate ester. Additionally, these effects are consistent with reports of decreased hunger, reduced energy intakes, and increased weight loss in subjects consuming low-carbohydrate ketogenic diets compared to low-fat diets or medium-carbohydrate non-ketogenic diets (McClernon *et al.,* 2007; Johnstone *et al.*, 2008).

Clinical chemistry analysis revealed that lactate dehydrogenase levels were significantly higher in ketone ester-fed rats (both sexes compared to control animals); however, the increases were small in magnitude and were not associated with changes in hemolysis parameters or histopathological effects. Microscopic findings (*e.g.*, vacuolation) in the liver were present in all groups and were not accompanied by effects on liver function parameters; therefore, they were not considered to be related to consumption of D-β-hydroxybutyrate ester.

Results from a developmental toxicity study in rats also support the safety of D-β-hydroxybutyrate ester (Clarke *et al.*, 2012b). Administration of 2 g D-β-hydroxybutyrate ester/kg body weight/day *via* gavage on Gestation Days 6 through 20 did not affect reproductive performance or litter parameters. Litter averages for corpora lutea, implantations, the percentage of pre-implantation loss, litter sizes, live and dead fetuses, early and late resorptions, the percentage of resorbed conceptuses, and the percentage of live male fetuses were comparable among groups. The overall incidence of fetal alterations (gross external, soft tissue, and skeletal combined) was significantly higher in the ketone ester group; however, there were no significant between-group differences in the litter or fetal incidences of any gross external, soft tissue, or skeletal abnormalities (malformations or variations) when assessed individually.

In a physical endurance study, adult men (mean age of 26 years) consumed a vitamin water drink containing 1.23 g D-β-hydroxybutyrate ester/kg body weight or 1.44 g dextrose/kg body weight (divided into 3 drinks of equal volume) before and during a cycling exercise session (Clarke *et al*., unpublished). D-β-Hydroxybutyrate ester was well tolerated under conditions similar to the intended conditions of use, with no significant differences in symptom severity between the D-β-hydroxybutyrate ester and control arms of the study. In another human study conducted in healthy male and female subjects 18 to 45 years of age, D-β-hydroxybutyrate ester was administered in a milkshake matrix at doses of 140, 357, and 714 mg/kg body weight 3 times daily (equivalent to 420, 1,071, and 2,142 mg/kg body weight/day) over a period of 5 days (Clarke *et al.*, 2012a). To standardize total daily caloric intake, subjects in the low- and mid-dose groups received Ensure®, a water-based beverage, as a supplemental formula instead of an equal volume of milkshake to the high-dose group. The ketone ester was generally

well tolerated, although some gastrointestinal effects (flatulence, nausea, diarrhea, constipation, vomiting, and abdominal distension and pain) were reported at the highest dose tested. In most instances, the effects were considered to be mild and occurred only once in each subject during the 5-day dosing period. These effects were attributed to the food matrix (milkshake) and dosing regimen (consumption of ~1.1 L within 1 minute) rather than to a toxicological effect of the test substance. Additionally, the taste of D-β-hydroxybutyrate ester, which is bitter and sharp, was likely a contributing factor to the reported nausea and vomiting. The reported gastrointestinal effects occurred at a lower frequency in individuals who consumed lower doses of D-β-hydroxybutyrate ester because the received a smaller volume of the test substance-containing milkshake. Consumption of Ensure® in the low- and mid-dose groups would not be expected to elicit the same gastrointestinal effects as an equal volume of milkshake. No abnormal changes in hematology, clinical biochemistry, or urinalysis parameters were observed. Moreover, levels of blood lipids, ketones, or glucose did not deviate from normal ranges.

The safety of D-β-hydroxybutyrate ester is supported by the results of studies conducted on its metabolites, D-β-hydroxybutyrate and (R)-1,3-butanediol. Sodium D,L-β-hydroxybutyrate has been administered orally to children with acyl-CoA dehydrogenase deficiency or with persistent hyperinsulinemic hypoglycemia at dose levels up to 1,000 mg/kg body weight/day with no side effects (Plecko *et al.,* 2002; Van Hove *et al.,* 2003). The results of *in vitro* studies (with isolated embryos) suggest that physiologically relevant levels of β-hydroxybutyrate, particularly the L-isomer, may disrupt normal embryogenesis (Sheehan *et al.,* 1985; Hunter *et al.,* 1987; Moley *et al.,* 1994); however, the results of an *in vivo* study with R,S-1,3-butanediol indicate that it is not teratogenic (Hess *et al.,* 1981). Because 100% and 30% of the R and S enantiomers, respectively, of 1,3-butanediol are metabolized to ketones (Desrochers *et al.*, 1992), studies on 1,3-butanediol indirectly provide information on the reproductive and developmental safety of β-hydroxybutyrate. Moreover, results of the rat developmental toxicity study on D-β-hydroxybutyrate ester indicated no adverse effects on development.

Almost all experimental studies on 1,3-butanediol identified administered both the (R)- and (S)-forms of butanediol, except for the chronic toxicity studies conducted by Scala and Paynter (1967). In this study, it was demonstrated that the 2-year oral administration of (R)-1,3-butanediol to Sprague-Dawley rats or to pure bred-beagles, at levels of 5 and 0.8 g/kg body weight/day, respectively, was not associated with toxicity. Hess *et al.* (1981) demonstrated, in 5 successive breedings of Wistar rats, that consumption of 5 to 24 g/kg body weight/day of 1,3-butanediol was not associated with developmental effects. The control and test groups were comparable with respect to gestation, viability, and lactation indices. The Acceptable Daily Intake established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for 1,3-butanediol is 4 mg/kg body weight/day (JECFA, 1980). Short-term human experimental studies indicate that 1,3-butanediol consumption may result in statistically, but not clinically significant reductions in blood glucose levels (Kies *et al.*, 1973; JECFA, 1980); however, it should be noted that no clinically significant effects on blood glucose levels were reported in the

5-day human study in which subjects consumed up to 2 g D-β-hydroxybutyrate ester/kg body weight/day (Clarke *et al.*, 2012a).

The safety of D-β-hydroxybutyrate ester is further supported by animal feeding trials of a similar ketone ester, namely (R,S)-1,3-butanediol mono- and diacetoacetate. A bolus intragastric dose of approximately 1.3 g/kg body weight administered to pigs was not associated with alterations in standard clinical chemistry parameters or with deleterious side effects (Desrochers *et al.,* 1995). Likewise, the administration of repeated oral doses of (R,S)-1,3-butanediol diacetoacetate over a 300-minute period (equivalent to 1,054 to 1,144 mg/kg body weight) or a single bolus dose of 439 to 477 mg/kg body weight (R,S)-1,3-butanediol diacetoacetate to dogs was not associated with alterations in clinical chemistry analyses; moreover, the authors reported no signs of distress in any of the animals during or following the experiment (Puchowicz *et al.,* 2000).

While D-β-hydroxybutyrate ester is a synthetic compound and thus, does not occur endogenously, the metabolites of D-β-hydroxybutyrate ester are ketones, which are produced in the body as a source of energy, particularly when blood glucose levels decrease. The normal 6- to 8-hour fasting blood ketone concentration of a healthy individual is approximately 0.5 mM, while after a 5- to 7-day fast, total blood ketone levels as high as 5 to 7 mM have been reported (Owen *et al.*, 1967; Mensink *et al.*, 1992; VanItalie and Nufert, 2003). In late pregnancy, blood ketone levels are reported to reach 4 to 6 mM after 48 hours of fasting (Robinson and Williamson, 1980). These levels are consistent with the maximum blood D-β-hydroxybutyrate levels reached (~4.0 mM) following consumption of the ketone ester under the intended conditions of use (Clarke *et al.*, unpublished). Additionally, maximal ketone body production is approximately 185 g/day during times of limited glucose availability such as fasting (Reichard *et al.*, 1974; Laffel, 1999), indicating that the human body has the capacity to handle large amounts of ketones and further corroborating the safety of D-β-hydroxybutyrate ester. Moreover, there is a long history of use of ketogenic diets. For example, they have been used for nearly a century in the treatment of pediatric refractory epilepsy (Wilder, 1921), and more recently for neurodegenerative diseases, including Alzheimer's (Reger *et al.*, 2004) and Parkinson's disease (VanItallie *et al.*, 2005). Variations of the ketogenic diet also have been investigated for their therapeutic effects on obesity (Dashti *et al.*, 2003, 2006; Yancy *et al.*, 2004), diabetes (Westman *et al.*, 2008; Al-Khalifa *et al.*, 2009), cancer (Freedland *et al.*, 2008; Otto *et al.*, 2008; Seyfried *et al.*, 2008), and a number of other conditions. Ketogenic diets elevate ketone levels, similar to D-β-hydroxybutyrate ester.

CONCLUSION

We, the Expert Panel, have independently and collectively, critically evaluated the data and information summarized above and unanimously conclude that the proposed uses of D-β-hydroxybutyrate ester as an ingredient to selected products targeted to high performing athletes for consumption on a supplemental basis, meeting appropriate food grade specifications and produced consistent with current good manufacturing practice (cGMP), are Generally Recognized as Safe (GRAS) based on scientific procedures,

It is our opinion that other qualified experts would concur with this conclusion.

(b) (6)

Professor Joseph F. Borzelleca, Ph.D.
Virginia Commonwealth University School of Medicine

29 July 2013
Date

(b) (6)

Professor Robert J. Nicolosi, Ph.D.
University of Massachusetts Lowell

26 July 2013
Date

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29 July 2013
Date

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Appendix A

Product Specifications for D-β-Hydroxybutyrate Ester

Table A-1 Chemical Specifications for D-β-Hydroxybutyrate Ester		
Specification Parameter	**Specification**	**Method**
D-β-hydroxybutyrate ester content	≥97.5%	GC/MS
Ethyl R 3-hydroxybutyrate content	<0.5%	GC/MS
R-1,3-butanediol content	<2.0%	GC/MS
Heavy Metals		
Mercury (Hg)	<0.008 mg/kg	UD030
Arsenic (As)	<0.1 mg/kg	UD031
Lead (Pb)	<0.005 mg/kg	UD032:ICPMS/005
Cadmium (Cd)	<0.005 mg/kg	UD033:ICPMS/005

GC/MS, gas chromatography/mass spectrometry; ICPMS, inductively coupled plasma mass spectrometry

Table A-2 Microbiological Specifications for D-β-Hydroxybutyrate Ester		
Specification Parameter	**Specification (CFU/mg)**	**Method**
Escherichia coli	< 5	HPA Standard Method F17, issue 2.4 May 2005
Moulds	< 10	BS 4285-3.6: 1986
Yeasts	< 10	BS 4285-3.6: 1986

BS = British Standards; CFU = colony forming unit; HPA = Health Protection Agency

Appendix B

Certificates of Analysis

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

www.nih.gov

CERTIFICATE OF ANALYSIS

FOR

1-(BETA-HYDROXYBUTYRYL) 1,3-BUTAN-DIOL (1-BHB)

LABORATORY OF METABOLIC CONTROL, NIAAA, NIH
RM 1S-22
5625 FISHERS LANE,
ROCKVILLE, MD 20892
USA

SAMPLE DESCRIPTION

CLEAR OIL PRODUCED FROM LIPASE REACTION (*C. ANTARTICA*) WITH **BETA-HYDROXY BUTYRATE ETHYL ESTER** AND 1,3- **BUTANE DIOL.**

SAMPLE ID : **delta_g_product_batch_7**
ORIGIN: OXFORD ENGLAND- RECEIVED NOVEMBER 2011

DATE ANALYZED

22/NOVEMBER/2011

METHOD OF ANALYSIS

DETERMINATION BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY. IONIZATION WITH THE 70 ELECTRON VOLT IN ELECTRON IMPACT MODE. SAMPLE OF THE OIL IS REACTED WITH TRI-METHYL SILYL ETHER FORMATION OF FREE HYDROXL GROUPS.

CONDITION

CLEAR OIL RECEIVED ON DRY-ICE IN SCREW-TOPPED NALGENE CONTAINERS, STORED AT -20^0 C UNTIL ANALYSIS.

ANALYTICAL PROCEDURES

GC-MS ANALYSIS

One uL of the sample was reacted with 20 uL of N, O-bis(Trimethylsilyl)trifluoroacetamide with 1% Trimethylchlorosilane for 2 min at 60^0. The sample was diluted with 200 uL of hexane and one uL was injected in the splitless mode onto a capillary DB-1 column (60 m) and the GC oven was heated from 60 to 235^0 at 15^o /min. The two mono esters (the 1- and 3- mono esters) eluted the column between 11.2 and 11.7 min. Analytes were ionized with 70eV of energy and the instrument was scanned from 50-800 amu.

SUMMARY OF ANALYSIS

Parameter	Specification	Result
β-hydroxybutyrate ester (includes both monoesters)	≥95%	98.83%
Ethyl hydroxybutyrate	≤1.5%	0.08%
R-1,3-butanediol	≤3.5%	0.73%

The sample contains approximately 98.83% of the 1-betahydroxybutyrl 1,3-butane diol monoesters with small amounts of starting materials ethyl hydroxybutyrate (0.08%) and R-1,3 butane diol (0.73 %).

(b) (6)

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(b) (6)

Robert J. Pawlosky, Ph.D.
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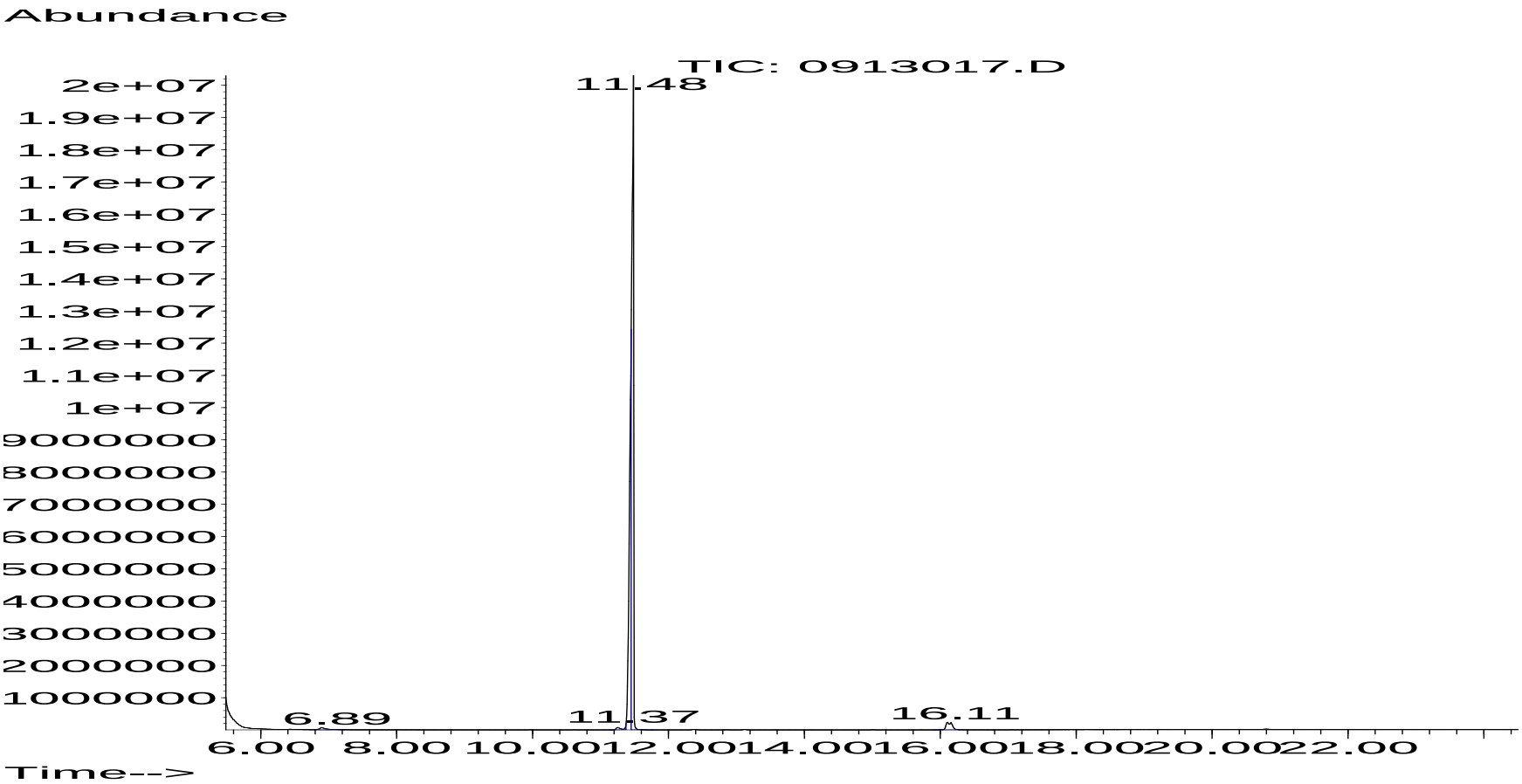
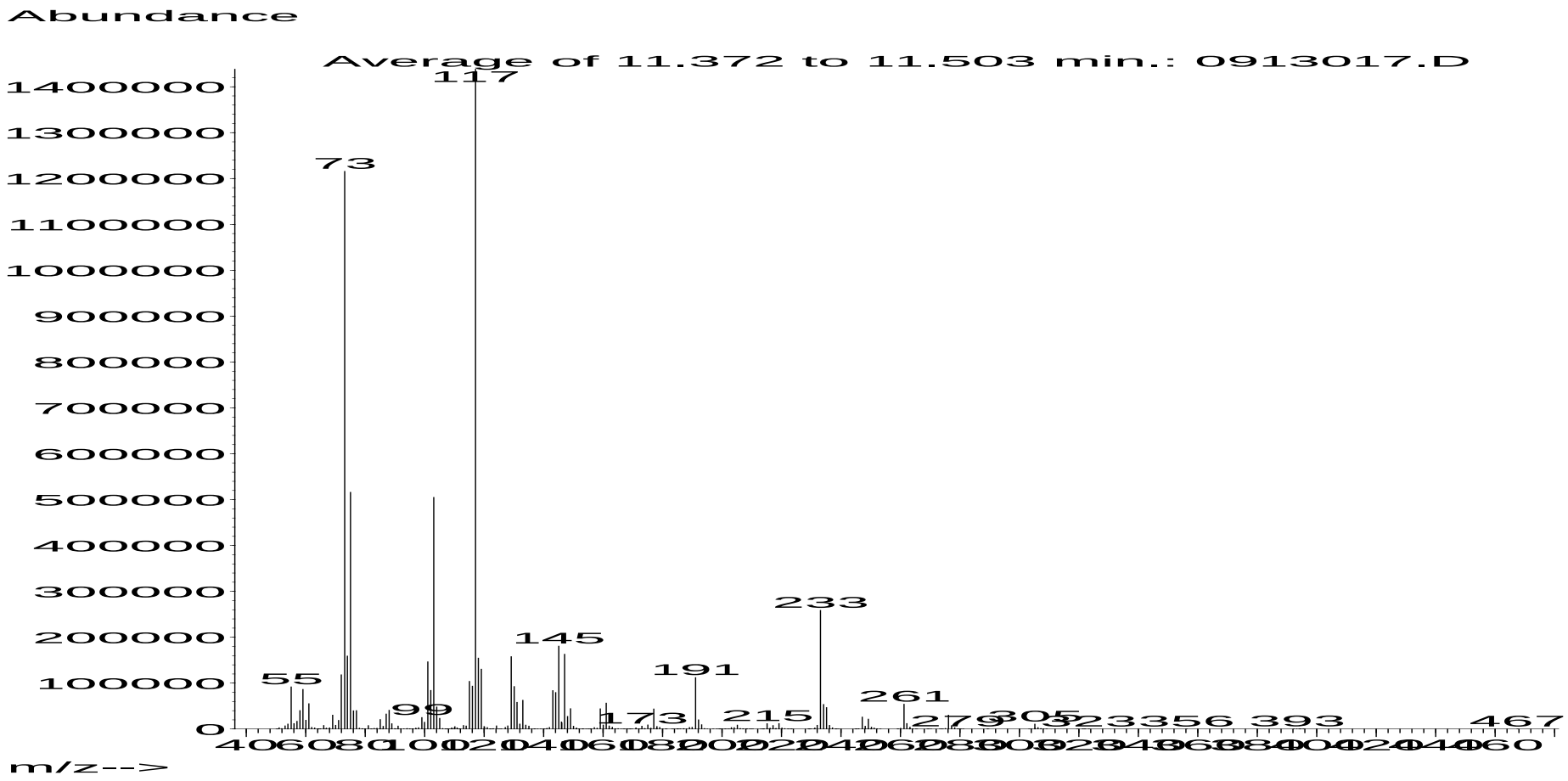
ANALYST

RESULTS OF ANALYSIS



Gas Chromatographic-mass spectral profile of beta-hydroxybutyrate 1,3 butane diol mono-ester products trimethylsilyl ethers produced from *C. antarctica* lipase from Oxford. Major product (98%) is the 1-BHB-1,3 butane diol mono-ester at 11.45 min.



70 eV EI mass spectrum the trimethylsilyl ether of the 1-betahydroxybytryl 1,3 butane diol mono-ester

CERTIFICATE OF ANALYSIS

FOR

1-(BETA-HYDROXYBUTYRYL) 1,3-BUTAN-DIOL (1-BHB)

LABORATORY OF METABOLIC CONTROL, NIAAA, NIH
RM 1S-22
5625 FISHERS LANE,
ROCKVILLE, MD 20892
USA

SAMPLE DESCRIPTION

CLEAR OIL PRODUCED FROM LIPASE REACTION (*C. ANTARTICA*) WITH **BETA-HYDROXY BUTYRATE ETHYL ESTER** AND 1,3- **BUTANE DIOL.**

SAMPLE ID : **delta_g_product_batch_8**
ORIGIN: OXFORD ENGLAND- RECEIVED, NOVEMBER 2011

DATE ANALYZED

22/NOVEMBER/2011

METHOD OF ANALYSIS

DETERMINATION BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY. IONIZATION WITH THE 70 ELECTRON VOLT IN ELECTRON IMPACT MODE. SAMPLE OF THE OIL IS REACTED WITH TRI-METHYL SILYL ETHER FORMATION OF FREE HYDROXL GROUPS.

CONDITION

CLEAR OIL RECEIVED ON DRY-ICE IN SCREW-TOPPED NALGENE CONTAINERS, STORED AT -20^0 C UNTIL ANALYSIS.

ANALYTICAL PROCEDURES

GC-MS ANALYSIS

One uL of the sample was reacted with 20 uL of N, O-bis(Trimethylsilyl)trifluoroacetamide with 1% Trimethylchlorosilane for 2 min at 60^0. The sample was diluted with 200 uL of hexane and one uL was injected in the splitless mode onto a capillary DB-1 column (60 m) and the GC oven was heated from 60 to 235^0 at 15^o /min. The two mono esters (the 1- and 3- mono esters) eluted the column between 11.2 and 11.7 min. Analytes were ionized with 70eV of energy and the instrument was scanned from 50-800 amu.

SUMMARY OF ANALYSIS

Parameter	Specification	Result
β-hydroxybutyrate ester (includes both monoesters)	≥95%	97.71%
Ethyl hydroxybutyrate	≤1.5%	0.12%
R-1,3-butanediol	≤3.5%	1.42%

The sample contains approximately 97.71% of the 1-betahydroxybutyrl 1,3-butane diol monoesters with small amounts of starting materials ethyl hydroxybutyrate (0.12%) and R-1,3 butane diol (1.42 %).

(b) (6)

Richard L. Veech, M.D., D. Phil.
Chief
Laboratory of Metabolic Control
NIAAA, National Institutes of Health

(b) (6)

Robert J. Pawlosky, Ph.D.
Laboratory of Metabolic Control
NIAAA, NIH
ANALYST

CERTIFICATE OF ANALYSIS

FOR

1-(BETA-HYDROXYBUTYRYL) 1,3-BUTAN-DIOL (1-BHB)

LABORATORY OF METABOLIC CONTROL, NIAAA, NIH
RM 1S-22
5625 FISHERS LANE,
ROCKVILLE, MD 20892
USA

SAMPLE DESCRIPTION

CLEAR OIL PRODUCED FROM LIPASE REACTION (*C. ANTARTICA*) WITH **BETA-HYDROXY BUTYRATE ETHYL ESTER** AND 1,3- **BUTANE DIOL.**

SAMPLE ID : **delta_g_product_batch_9**
ORIGIN: OXFORD ENGLAND- RECEIVED, NOVEMBER 2011

DATE ANALYZED

22/NOVEMBER/2011

METHOD OF ANALYSIS

DETERMINATION BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY. IONIZATION WITH THE 70 ELECTRON VOLT IN ELECTRON IMPACT MODE. SAMPLE OF THE OIL IS REACTED WITH TRI-METHYL SILYL ETHER FORMATION OF FREE HYDROXL GROUPS.

CONDITION

CLEAR OIL RECEIVED ON DRY-ICE IN SCREW-TOPPED NALGENE CONTAINERS, STORED AT -20^0 C UNTIL ANALYSIS.

ANALYTICAL PROCEDURES

GC-MS ANALYSIS

One uL of the sample was reacted with 20 uL of N, O-bis(Trimethylsilyl)trifluoroacetamide with 1% Trimethylchlorosilane for 2 min at 60^0. The sample was diluted with 200 uL of hexane and one uL was injected in the splitless mode onto a capillary DB-1 column (60 m) and the GC oven was heated from 60 to 235^0 at 15^o /min. The two mono esters (the 1- and 3- mono esters) eluted the column between 11.2 and 11.7 min. Analytes were ionized with 70eV of energy and the instrument was scanned from 50-800 amu.

SUMMARY OF ANALYSIS

Parameter	Specification	Result
β-hydroxybutyrate ester (includes both monoesters)	≥95%	98.19%
Ethyl hydroxybutyrate	≤1.5%	0.04%
R-1,3-butanediol	≤3.5%	0.78%

The sample contains approximately 98.19% of the 1-betahydroxybutyrl 1,3-butane diol monoesters with small amounts of starting materials ethyl hydroxybutyrate (0.04%) and R-1,3 butane diol (0.78%).

(b) (6)

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Chief
Laboratory of Metabolic Control
NIAAA, National Institutes of Health

(b) (6)

Robert J. Pawlosky, Ph.D.
Laboratory of Metabolic Control
NIAAA, NIH
ANALYST

PO Number **None Supplied**

Emma Carter
University of Oxford
Cardiac Metabolism
Research Group
Department of Physiology,
Anatomy & Genetics
Parks Road
Oxford
OX1 3PT

AR-11-UD-142816-01

Reported on **21/11/2011**
Reported by **Alan Cadman, Analytical Services Manager**

Certificate Of Analysis

Sample number	**400-2011-20075084**	Received on	**17/11/2011**
Your sample reference	**Delta G Batch 7 (15mls)**	Your sample code	**Delta G Batch 7 (15mls)**

Test Code	Analyte	Result		SOP No.
Toxic Elements				
UD401	Arsenic	0.012	mg/kg	ICPMS/010
UD033	Cadmium	<0.001	mg/kg	ICPMS/010
UD032	Lead	<0.005	mg/kg	ICPMS/010
UD579	Mercury	<0.001	mg/kg	ICPMS/010

Unless stated, all results are expressed on a sample as received basis.
† Indicates that this test was subcontracted
italicised parameters indicate accreditation under flexible scope
* Indicates that this parameter is not included in the UKAS accreditation schedule for the laboratory.
Opinions and/or interpretations within this report are outside our accreditation scope.

PO Number **None Supplied**

Emma Carter
University of Oxford
Cardiac Metabolism
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Parks Road
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OX1 3PT

AR-11-UD-142817-01

Reported on **21/11/2011**
Reported by **Alan Cadman, Analytical Services Manager**

Certificate Of Analysis

Sample number	**400-2011-20075087**	Received on	**17/11/2011**
Your sample reference	**Delta G Batch 8 (15mls)**	Your sample code	**Delta G Batch 8 (15mls)**

Test Code	Analyte	Result		SOP No.
Toxic Elements				
UD401	Arsenic	0.013	mg/kg	ICPMS/010
UD033	Cadmium	<0.001	mg/kg	ICPMS/010
UD032	Lead	<0.005	mg/kg	ICPMS/010
UD579	Mercury	<0.001	mg/kg	ICPMS/010

Unless stated, all results are expressed on a sample as received basis.
† Indicates that this test was subcontracted
italicised parameters indicate accreditation under flexible scope
* Indicates that this parameter is not included in the UKAS accreditation schedule for the laboratory.
Opinions and/or interpretations within this report are outside our accreditation scope.

PO Number **None Supplied**

Emma Carter
University of Oxford
Cardiac Metabolism
Research Group
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Anatomy & Genetics
Parks Road
Oxford
OX1 3PT

AR-11-UD-142815-01

Reported on **21/11/2011**
Reported by **Alan Cadman, Analytical Services Manager**

Certificate Of Analysis

Sample number	**400-2011-20075090**	Received on	**17/11/2011**
Your sample reference	**Delta G Batch 9 (15mls)**	Your sample code	**Delta G Batch 9 (15mls)**

Test Code	Analyte	Result		SOP No.
Toxic Elements				
UD401	Arsenic	0.011	mg/kg	ICPMS/010
UD033	Cadmium	<0.001	mg/kg	ICPMS/010
UD032	Lead	<0.005	mg/kg	ICPMS/010
UD579	Mercury	<0.001	mg/kg	ICPMS/010

Unless stated, all results are expressed on a sample as received basis.
† Indicates that this test was subcontracted
italicised parameters indicate accreditation under flexible scope
* Indicates that this parameter is not included in the UKAS accreditation schedule for the laboratory.
Opinions and/or interpretations within this report are outside our accreditation scope.

Memorandum of to the File

Date: April 30, 2014

To: GRN 515 - (R)-3-hydroxybutyl (R)-3-hydroxbutyrate

Subject: Revised GRAS Exemption Claim with live signature - Filing Date

FDA received a revised GRAS exemption claim from Professor Kieran Clarke, of TdeltaS Limted regarding their GRAS determination for their submission.

The appropriate filing date is April 30, 2014, the date of the receipt of the amendment rendering the submission fillable as a GRAS notice.

Richard E. Bonnette

Cc: GRN 000515
FT:HFS-255:RBonnette:HFS-255:4/30/14

Richard E. Bonnette Iii III -S	Digitally signed by Richard E. Bonnette Iii III -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300212202, cn=Richard E. Bonnette Iii III -S Date: 2014.04.30 12:55:16 -04'00'	

Bonnette, Richard

From: Kieran Clarke <kieran.clarke@dpag.ox.ac.uk>
Sent: Friday, April 25, 2014 2:32 AM
To: Bonnette, Richard
Cc: Nicola Chubb; Ashley Roberts Intertek (ashley.roberts@intertek.com)
Subject: RE: your GRAS notice for (R)-3-hydroxybutyl (R)-3-hydroxybutyrate

Dear Richard,

Many thanks for your email to let me know that my signature on the GRAS exemption claim on page 1 should be an original. I will sign the page and send it to you immediately.

Thank you again and kind regards,
Kieran Clarke

Professor Kieran Clarke
University of Oxford
Department of Physiology, Anatomy & Genetics
Sherrington Building
Parks Road
Oxford OX1 3PT
UK
Telephone: +44 (0) 1865 282248
Facsimile: +44 (0) 1865 282272

From: Bonnette, Richard [mailto:Richard.Bonnette@fda.hhs.gov]
Sent: 24 April 2014 15:38
To: Kieran Clarke
Subject: your GRAS notice for (R)-3-hydroxybutyl (R)-3-hydroxybutyrate

Professor Clarke,
I have been asked to conduct a pre-filing review of your submission to the US FDA's GRAS Notification program that was recently received by our office. There is a minor administrative issue that is preventing its filing relating to the signature on the submission. The signature on the GRAS exemption claim on page 1 appears to be a photocopy or a reproduction. We do request that the signature for the GRAS exemption be original. The easiest remedy is to provide a replacement page that is physically signed, sent to my attention. Once we have the signed and dated exemption claim, the notice will be filed. Please let me know if you have any questions, or if anything here is unclear.

Kind regards,
Richard

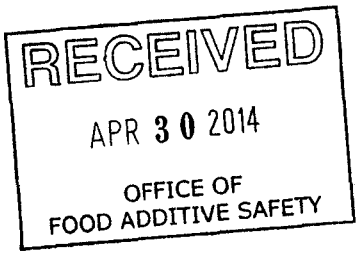
Richard E. Bonnette, M.S.
Consumer Safety Officer
Office of Food Additive Safety, Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration

Mail:

Division of Biotechnology and GRAS Notice Review (HFS-255)
5100 Paint Branch Parkway
College Park, MD 20740

Direct: 240-402-1235
Richard.Bonnette@fda.hhs.gov

Please find enclosed covering letter + exemption claim, both with original signatures.



WITH COMPLIMENTS

(b) (6)

PA to Prof Kieran Clarke.

Professor Kieran Clarke, Professor of Physiological Biochemistry
Department of Physiology, Anatomy & Genetics, Sherrington Building, Parks Road, Oxford, OX1 3PT
Tel: 01865 282248 Fax: 01865 282272 Email: kieran.clarke@tdeltas.co.uk

TdeltaS Limited
Registered Office:
30 Upper High Street
Thame
Oxfordshire OX9 3EZ

Email: *Kieran.Clarke@dpag.ox.ac.uk*
Telephone: *+44 1865 282246*
Fax: *+44 1865 282272*

April 25, 2014

Dr Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Dr Gaynor:

Re: GRAS Exemption Claim for (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized as Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting one hard copy and one electronic copy (on CD), as the notifier [TdeltaS Limited, University of Oxford, Parks Road, Oxford, United Kingdom], a notice of the determination, on the basis of scientific procedures, that (R)-3-hydroxybutyl (R)-3-hydroxybutyrate (D-β-hydroxybutyrate ester), as defined in the enclosed documentation, is GRAS under specific conditions of use as a food ingredient for use in foods consumed exclusively by high-performance athletes and individuals undergoing high energy expenditure and, therefore, is exempt from the pre-market approval requirements of the *Federal, Food, Drug and Cosmetic Act*. Information setting forth the basis for the GRAS determination, which includes detailed information on the notified substance and a summary of the basis for the GRAS determination, as well as a consensus opinion of an independent panel of experts in support of the safety of D-β-hydroxybutyrate ester under the intended conditions of use, also are enclosed for review by the Agency.

The enclosed electronic file for the Notice entitled, "GRAS Exemption Claim for (R) 3 hydroxybutyl (R)-3-hydroxybutyrate" was scanned for viruses prior to submission and is thus certified as being virus-free using McAfee VirusScan 8.8.

Should you have any questions or concerns regarding this GRAS Notification, please do not hesitate to contact me at any point during the review process, so that we may provide a response in a timely manner.

Sincerely,

(b) (6)

Professor Kieran Clarke
TdeltaS Limited
University of Oxford, UK

I GRAS EXEMPTION CLAIM

I.A Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997) (U.S. FDA, 1997)]

(*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate (D-β-hydroxybutyrate ester) has been determined to be Generally Recognized as Safe (GRAS) by TΔS Limited (TΔS) for use as a food ingredient in the United States (U.S.), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections. Therefore, the use of D-β-hydroxybutyrate ester in foods as described below is exempt from the requirement of premarket approval.

Signed,

(b) (6)

25 April 2014.

Professor Kieran Clarke
Director

Date

I.B Name and Address of Notifier

Professor Kieran Clarke
TΔS Limited
University of Oxford
Parks Road, Oxford
United Kingdom
OX1 3PT

I.C Common Name of the Notified Substance

The common name of the notified substance is D-β-hydroxybutyrate ester.

I.D Conditions of Intended Use in Food

I.D.1 Intended Uses of D-β-Hydroxybutyrate Ester and Levels of Use

TΔS proposes to market D-β-hydroxybutyrate ester in selected categories of products (beverages, bars, and gels) designed exclusively for high-performance athletes and individuals undergoing extreme energy expenditure. D-β-hydroxybutyrate ester will be added to these

SUBMISSION END